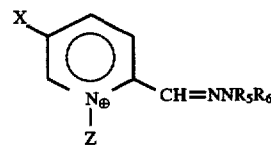




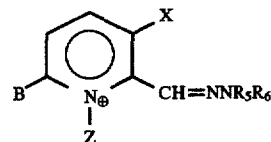
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United States Patent [19]**Powers et al.**[11] **Patent Number:** **5,726,314**[45] **Date of Patent:** **Mar. 10, 1998**[54] **PYRIDINIUM COMPOUNDS**[75] **Inventors:** **James C. Powers**, Atlanta; **Jerry J. Buccafusco**, Evans; **Kenneth M. Starks**, Atlanta, all of Ga.[73] **Assignee:** **Georgia Tech Research Corp.**, Atlanta, Ga.[21] **Appl. No.:** **723,293**[22] **Filed:** **Sep. 30, 1996****Related U.S. Application Data**[62] **Division of Ser. No. 193,217**, Feb. 8, 1994.[51] **Int. Cl.⁶** **C07D 213/75**; C07D 213/65;
C07D 213/72[52] **U.S. Cl.** **546/292**; 546/303; 546/330;
546/331; 546/291[58] **Field of Search** 546/291, 292,
546/303, 330, 331[56] **References Cited****U.S. PATENT DOCUMENTS**5,180,831 1/1993 Powers et al. 546/291
5,206,371 4/1993 Powers et al. 546/290**Primary Examiner**—Alan L. Rotman**Attorney, Agent, or Firm**—Deveau, Colton & Marquis[57] **ABSTRACT**

Novel quaternary pyridinium compounds useful in inhibiting acetylcholinesterase in mammals of the general structure:



and



or pharmaceutically acceptable salts thereof, where R_5 is pentafluorophenyl, phenyl monosubstituted with K, phenyl disubstituted with K, phenyl trisubstituted with K, or naphthyl; R_6 is H, C_{1-6} alkyl, phenyl, pentafluorophenyl, phenyl mono, di, or trisubstituted with K, C_{1-6} alkyl with an attached phenyl group, C_{1-6} alkyl with an attached phenyl group mono, di, or trisubstituted with K; K is halogen, COOH, OH, CN, NO_2 , NH_2 , C_{1-6} alkyl, C_{1-6} fluorinated alkyl, C_{1-6} alkoxy, C_{1-6} alkylamine, C_{1-6} alkyl—O—CO—, C_{1-6} alkyl—CO—NH—, C_{1-6} alkyl—NH—CO—NH—, C_{1-6} alkyl—NH—CO—O—, C_{1-6} alkyl—NH—CO—, or $(C_{1-6} \text{ alkyl})_2N—CO—$; X is OH, C_{1-6} alkyl—NH—CO—O—, $(C_{1-6} \text{ alkyl})_2N—CO—O—$, C_{1-6} fluoroalkyl—NH—CO—, or $(C_{1-6} \text{ fluoroalkyl})_2N—NH—CO—O—$; and B is H or C_{1-6} alkyl.

1 Claim, No Drawings

PYRIDINIUM COMPOUNDS

This is a divisional of copending application Ser. No. 08/193,217 filed on Feb. 8, 1994.

STATEMENT OF GOVERNMENT INTEREST

This invention was made with Government support under Contract No. DAMD17-85-C-5143 awarded by the U.S. Army Medical Research and Development Command. The Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

1. Field of the Invention

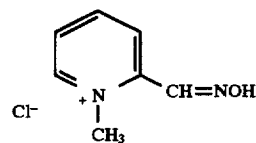
This invention relates to a novel class of quaternary pyridinium compounds useful for selectively inhibiting acetylcholinesterase (AChE) and to their use as nerve agent antidotes and prophylactics. We have found that certain quaternary pyridinium derivatives can offer in vivo protection against the nerve agents Soman (GD) and Tabun (CA) and therefore they can also offer protection against other organophosphate poisons. We have also found that certain quaternary pyridinium derivatives can effectively increase the rate of survival of experimental animals exposed to the nerve agents GD and GA and therefore they can also serve as antidotes against other organophosphate poisons. All of the active pyridinium compounds are good AChE inhibitors in vitro and therefore they can also be used to treat conditions where a cholinergic deficit leading to pathology can be ameliorated by administration of an anticholinesterase agent. All of the pyridinium compounds described herein can have additional beneficial effects in living organisms by virtue of their resemblance to the natural neurotransmitter acetylcholine and their ability to act on cholinergic receptors. We have also found that the pyridinium compounds can be used to treat Alzheimer's disease (senile dementia), presenile dementia of the Alzheimer's type, Korsakoffs syndrome (psychosis), age-related memory impairment, multi-infarct dementia, Parkinsonian dementia, Down's syndrome, and posttraumatic dementia (dementia pugilistica).

2. Description of the Related Art

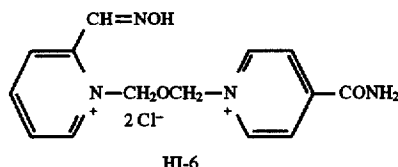
Development of an effective antidote to organophosphorus (OP) nerve agents has been a goal of medicinal chemists since the development of Tabun in Germany in 1937. OP agents react covalently with the active site serine hydroxyl group of acetylcholinesterase (AChE) to form a stable phosphonyl ester which requires up to 30 days for hydrolysis (Main, A. R. In *Biology of Cholinergic Function*; Goldberg, A. M., Hanin, I., Eds.; Raven Press: New York, 1976; pp. 269-353, incorporated herein by reference). Excess acetylcholine accumulates and causes severe neurological imbalance, respiratory paralysis, and death (Koelle, G. B. In *The Pharmacological Basis of Therapeutics*; Goodman, L., and Gilman, A., Eds.; MacMillan: New York, 1975; p. 404, incorporated herein by reference). pyridinium aldoximes (2-PAM and HI-6, see structures below) are currently used in conjunction with an acetylcholine antagonist (atropine) to reactivate the phosphonylated serine hydroxyl group of AChE after poisoning occurs (Leadbeater, L., Inns, R. H., and Rylands, J. M. *Fund. Appl. Toxicol.* 1985, 5, S225, incorporated herein by reference). This treatment is ineffective against GD and GA because the initially formed enzyme bound alkyl phosphonate esters undergo rapid aging with loss of the alkoxy side chain to form a negatively charged phosphonyl mono ester (Wolthuis, O. L., Berends, F., and Meeter, E., *Fund. Appl. Toxicol.* 1981, 1, 183, incorporated

herein by reference). The negative charge on this mono ester repels nucleophiles which are required to dephosphonylate the enzyme.

Other approaches to OP antidotes have dealt with changes in the heteroaromatic ring system in order to improve the reactivation of OP-inhibited acetylcholinesterase (Bedford, C. D., Harris, R. N., III, Howd, R. A., Goff, D. A., Koolpe, G. A., Petesch, M., Koplovitz, I., Sultan, W. E., and Musallam, H. A., *J. Med. Chem.* 1989, 32, 504; Bedford, C. D., Harris, R. N., Howd, R. A., Goff, D. A., Koolpe, G. A., Petesch, M., Miller, A., Nolen, H. W., III, Musallam, H. A., Pick, R. O., Jones, D. E., Koplovitz, I., and Sultan, W. E., *J. Med. Chem.* 1989, 32, 493; Benschop, H. P., Van der Berg, G. R., Van der Hooijdonk, C., DeJong, L. P. A., Kientz, C. E., Berends, F., Kepner, L. A., Meeter, E., and Visser, R. P. L. S., *J. Med. Chem.* 1979, 22, 1306; Grifantini, M., Martelli, S., and Stein, M. L., *J. Med. Chem.* 1973, 16, 937, incorporated herein by reference).



2-PAM Chloride



HI-6

Since it is difficult to reactivate aged phosphonylated AChE, prophylactic treatment with AChE inhibitors may be the preferred alternative. AChE is protected from nerve agents by preadministration of an AChE carbamylating agent such as pyridostigmine or physostigmine. These carbamates react covalently with AChE at the active site serine, but the carbamylated enzyme is hydrolyzed quickly to active enzyme (30 min.) while phosphonylated AChE requires several hours or days for regeneration of active AChE (Main, A. R., vide supra). Blocking AChE for a short period of time allows destruction of the OP agent by uncatalyzed hydrolysis or reaction with enzymes such as carboxylesterase or pseudocholinesterase (Harris, L. W., Stücher, D. L., and Heyl, W. C., *Life Sci.* 1980, 26, 1885, incorporated herein by reference). Unfortunately, physostigmine is very toxic and small doses can be lethal. Furthermore, physostigmine is unstable and would rapidly decompose in the battlefield. Both carbamates are effective only when combined with several other drugs (Heyl, W. C., Harris, L. W., and Stücher, D. L., *Drug Chem. Toxicol.* 1980, 3, 319, incorporated herein by reference).

Other approaches to prophylaxis against OP poisoning have involved the use of cyclic organophosphates to temporarily block AChE (Ashani, Y., Leader, H., Raveh, L., Bruckstein, R. and Spiegelstein, M., *J. Med. Chem.* 1983, 26, 145, incorporated herein by reference), reduction of AChE levels by inhibition of choline acetyltransferase (Gray, A. P., Platz, R. D., Henderson, T. R., Chang, T. C. P., Takahashi, K. and Dretchen, K. L., *J. Med. Chem.* 1988, 31, 807, incorporated herein by reference), and the use of antimuscarinic α -adrenergic agonists (e.g., clonidine) to block the release of acetylcholine from presynaptic nerve terminals (Buccafusco, J. J. and Aronstam, R. S., *J. Pharmacol. Exp. Ther.* 1986, 239, 43, incorporated herein by reference).

Many oxime, semicarbazone, hydrazone and acyl hydrazone derivatives of 2-formyl-3-hydroxy-1-methylpyridinium chloride described herein incorporate both a nucleophile to displace the phosphonate from the serine hydroxyl group of AChE and an additional hydrogen bonding group (hydroxyl) to neutralize the negative charge on the aged phosphonate mono ester. In addition, the parent 3-hydroxypyridinium derivatives were converted into various carbamates since these are pyridostigmine analogs and can react covalently with AChE and thus provide protection from OP agents.

Treatment of dementing illnesses such as Alzheimer's Disease (AD) represents a significant clinical challenge. While several palliative treatments have been attempted, pharmacological interventions which enhance central cholinergic neurotransmission have often provided limited beneficial effects. However, serious limitations with regard to long-term therapy with cholinergic agonists include narrow therapeutic windows, tolerance to the beneficial effects, severe side effects and unpredictable patient response to standard dosing regimens. Consequently, there is considerable interest in the development of new therapeutic agents. With greater efficacy and improved selectivity. One approach which has proven successful is the development of novel compounds based upon the results from studies employing known pharmacological agents or combinations of agents. From a therapeutic standpoint, it would be best if the same molecule could target multiple sites or act through multiple mechanisms. Part of the basis for these concepts derives from the nature of the disease itself.

Post mortem studies of AD have demonstrated a loss of markers specific for brain cholinergic neurons. While the cholinergic loss in AD has a clear basis in the known amnesic actions of cholinergic (muscarinic) antagonists, it is known that several other neurotransmitters and related growth factors may also be affected. For example, evidence has been accumulating from a number of studies supporting the existence of functional nicotinic receptors in the brain. Of recent interest has been its role in normal and pathologic cognitive processing. Post mortem brain tissue receptor binding studies in AD have demonstrated significant abnormalities of the nicotinic cholinergic system. The nucleus basalis, located in the ventromedial corner of the globus pallidus, is part of the basal forebrain cholinergic system which provides the major source of extrinsic cholinergic input to the frontal cortex, and is known to be involved in neuronal processes mediating cognitive function and integration at the highest level. Interestingly, this region is known to undergo extensive degeneration in AD. While the functional role of such nicotinic receptors is unknown, one possibility is that they may serve as a positive feedback mechanism for acetylcholine release. Using the prototype agonists nicotine, it has been demonstrated that stimulation of central nicotinic receptors produces a significant and reliable increase in performance of the automated delayed matching-to-sample (DMTS) task by both young and aged monkeys (Buccafusco, J. J. and Jackson, W. J., Beneficial effects of nicotine administered prior to a delayed matching-to-sample task in young and aged monkeys, *Neurobiol. Aging* 12:233-238 (1991)). More recently one of the inventors has demonstrated that muscarinic receptors mediate the enhanced DMTS performance, reflecting the acetylcholine releasing action of nicotine (Terry, A. V., Buccafusco, J. J. and Jackson, W. J., Scopolamine reversal of nicotine enhanced delayed matching-to-sample performance in monkeys, *Pharmacol. Biochem. Behav.* 45:925-929 (1993)). The possibility exists, therefore, that nicotinic receptors may

be associated with, or located on, cholinergic fibers projecting to the cortical regions which are involved in AD. Unfortunately, nicotine, which is an extremely toxic substance, is one of the few agonists of the neuronal nicotinic receptor that produces central actions following peripheral administration. In order to pharmacologically exploit central nicotinic receptors it will be necessary to develop new centrally acting nicotinic agonists with less side effects and toxicity than nicotine.

A superior approach might be to combine the pharmacological properties of central AChE inhibition with nicotinic receptor activation. In addition to providing two potential cognitive enhancing mechanisms to target, the presynaptic down-regulation (reduced transmitter release) produced during chronic esterase inhibition might be avoided through stimulation of nicotinic receptors located on nerve endings which normally enhance acetylcholine release. To this end, we have synthesized pyridinium compounds, compounds designed primarily as specific inhibitors of AChE. These compounds are different from other previous centrally-acting AChE inhibitors used to treat Alzheimer's patients in that the molecule retains a permanent positive charge and therefore, may exhibit the ability to directly stimulate cholinergic receptors as well as inhibit the esterase. The ability of these compounds to cross the blood-brain barrier is due to their enhanced hydrophobicity. In this invention, we show that it is possible to develop centrally-acting drugs with permanent positive charges. Such compounds exhibit the ability to both inhibit cholinesterase and stimulate nicotinic (and/or muscarinic) receptors in the central nervous system.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a graph showing the effect of MHP-133 (4b) on DMTS performance as a function of dose. Data are presented for Long (upper panel) and Medium (lower panel) delay intervals. Each value represents mean+S.E.M. from 5 monkeys. The numbers in the upper panel indicate the numbers of animals contributing data within the doses bracketed by the vertical axes.

FIG. 2 is a graph showing the effect of MHP-133 (4b) on DMTS performance as a function of dose. Data are presented for Long (filled bars) and Medium (open bars) delay intervals. Each value represents mean+S.E.M. from 11 monkeys. The * indicates a significant increase ($P<0/05$) with regard to control (vehicle) performance levels.

FIG. 3 is a graph showing the effect of MHP-133 (4b) on DMTS performance in 11 monkeys as a function of delay interval. "Best Dose" refers to that dose selected (between 30-100 $\mu\text{g/kg}$, inclusive) for each individual animal which provided the best overall (all delays combined) improvement in performance. The * indicates a significant increase ($P<0/05$) with regard to control (vehicle) performance levels.

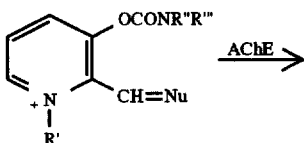
FIG. 4 is a graph showing the effect of 3 doses of MHP-133 (4b) on the performance in 4 trials of the Morris Water Maze task by young mature rats. Each point represents the mean+S.E.M. from 10 rats. Latency indicates the time interval between placing the rat in the maze and his locating the hidden platform. The * indicates a significant decrease ($P<0/05$) with regard to control (vehicle) latencies.

DETAILED DESCRIPTION OF THE INVENTION

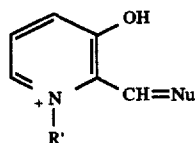
Aldoxime, hydrazone, semicarbazone and acyl hydrazone derivatives of 2-formyl-1-substituted pyridinium compounds are good inhibitors of acetylcholinesterase (ACE)

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and can displace a nerve agent from the enzyme active site or protect the latter from attack by a nerve agent. Therefore these structures can be used as antidotes and prophylactics against nerve agents or other organophosphate poisons. Furthermore, it is possible that the carbamates act first as prophylactics against OP poisoning and that the reaction products (3-hydroxy derivatives) act as reactivators of phosphorylated AChE (see below). Herein we describe one instance of this prophylactic/prodrug concept.



Prophylactic

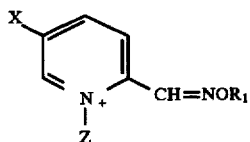


Reactivator

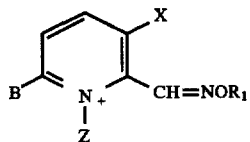
Nu = nucleophile

In addition to their AChE inhibitory properties, these derivatives can act on cholinergic receptors in the nervous system either to mimic or to oppose the effects of the natural neurotransmitter acetylcholine. Therefore these structures can also be used in the treatment of conditions such as Myasthenia Gravis, Alzheimer's disease (senile dementia), presenile dementia of the Alzheimer's type, Korsakoffs syndrome (psychosis), age-related memory impairment, multi-infarct dementia, Parkinsonian dementia, Down's syndrome, and posttraumatic dementia (dementia pugilistica) where a cholinergic deficit leading to pathology can be ameliorated by administration of a cholinomimetic or an anticholinesterase agent. These structures can also act on the cardiovascular system to produce an antihypertensive effect; they can be useful in the treatment of glaucoma; they can enhance bowel tone and motility.

The novel substituted pyridinium derivatives have the following structural formulas:



and



with any counterion to make pharmaceutically acceptable salts, wherein

Z is selected from the group consisting of C₁₋₆ alkyl, C₁₋₆ alkyl with an attached phenyl group, C₁₋₆ alkyl with an attached naphthyl group, C₁₋₆ alkyl with two attached phenyl groups, C₁₋₆ alkyl with an attached phenyl group mono, di, or trisubstituted with J, C₁₋₆ alkyl with two attached phenyl

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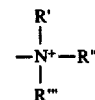
groups mono, di, or trisubstituted with J, C₁₋₆ alkyl with an attached naphthyl group mono, di, or trisubstituted with J,

wherein J is selected from the group consisting of halogen, COOH, OH, CN, NO₂, NH₂, C₁₋₆ alkyl, C₁₋₆ alkoxy, C₁₋₆ alkylamine, C₁₋₆ alkyl-O-CO-, C₁₋₆ alkyl-O-CO-NH-, C₁₋₆ alkyl-NH-CO-NH-, C₁₋₆ alkyl-NH-CO-O-, C₁₋₆ alkyl NH-CO-, (C₁₋₆alkyl)₂N-CO-,

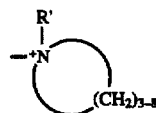
wherein X is selected from the group consisting of OH, C₁₋₆ alkyl-NH-CO-O-, (C₁₋₆ alkyl)₂N-CO-O-, C₁₋₆ fluoroalkyl-NH-CO-O-, (C₁₋₆ fluoroalkyl)₂NH-CO-O-,

wherein R₁ is selected from the group consisting of C₁₋₆ alkyl, C₁₋₆ alkyl with an attached phenyl group, C₁₋₆ alkyl with two attached phenyl groups, C₁₋₆ alkyl with an attached phenyl group mono, di, or trisubstituted with K, C₁₋₆ alkyl with two attached phenyl groups mono, di, or trisubstituted with K, C₁₋₆ alkyl with an attached naphthyl group, C₁₋₆ alkyl with an attached naphthyl group mono, di, or trisubstituted with K, C₁₋₆ alkyl-T,

wherein T is selected from the group consisting of formula (I) and formula (II).



Formula I



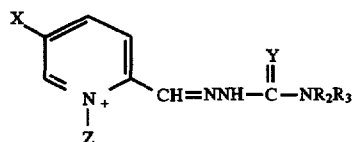
Formula II

wherein R', R'', and R''' are selected from the group consisting of H, C₁₋₆ alkyl, C₁₋₆ fluoroalkyl, C₁₋₆ alkyl with an attached phenyl group, C₁₋₆ alkyl with an attached phenyl group mono, di, or trisubstituted with K, C₁₋₆ fluoroalkyl with an attached phenyl group mono, di, or trisubstituted with K,

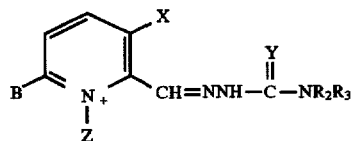
wherein K is selected from the group consisting of halogen, COOH, OH, CN, NO₂, NH₂, C₁₋₆ alkyl, C₁₋₆ alkoxy, C₁₋₆ alkylamine, C₁₋₆ alkyl-O-CO-, C₁₋₆ alkyl-O-CO-NH-, C₁₋₆ alkyl-NH-CO-NH-, C₁₋₆ alkyl-NH-CO-O-, C₁₋₆ alkyl NH-CO-, (C₁₋₆ alkyl)₂N-CO-,

wherein B is selected from the group consisting of H, C₁₋₆ alkyl.

Alternatively the novel quaternary pyridinium compounds are represented by the following structural formulas:



and



with any counterion to make pharmaceutically acceptable salts, wherein

Y is O or S.

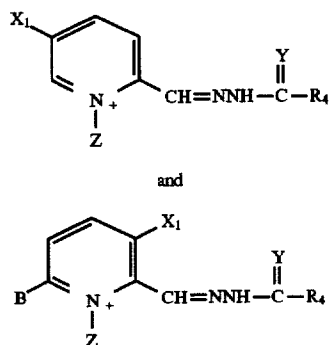
wherein R₂ is selected from the group consisting of H, C₁₋₆ alkyl, phenyl, C₁₋₆ alkyl with an attached phenyl

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group, C₁₋₆ alkyl with an attached phenyl group mono, di, or trisubstituted with K, C₁₋₆ alkyl with an attached naphthyl group, C₁₋₆ alkyl with an attached naphthyl group mono, di, or trisubstituted with K.

wherein R₃ is selected from the group consisting of H, C₁₋₆ alkyl, phenyl, C₁₋₆ alkyl with an attached phenyl group, C₁₋₆ alkyl with an attached phenyl group mono, di, or trisubstituted with K.

Alternatively the novel quaternary pyridinium compounds are represented by the following structural formulas:

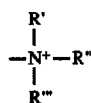


with any counterion to make pharmaceutically acceptable salts, wherein

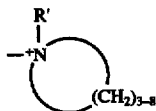
X₁ is selected from the group consisting of H, OH, C₁₋₆ alkyl—NH—CO—O—, (C₁₋₆ alkyl)₂—N—CO—O—, C₁₋₆ fluoroalkyl—NH—CO—O—, (C₁₋₆ fluoroalkyl)₂—NH—CO—O—,

wherein R₄ is selected from the group consisting of C₁₋₆ alkyl with a pyridinium ring attached through nitrogen, C₁₋₆ alkyl with a pyridinium ring attached through nitrogen and mono, di, or trisubstituted with K, C₁₋₆ alkyl with a quolinium ring attached through nitrogen and mono, di, or trisubstituted with K, C₁₋₆ alkyl with an isoquinolinium ring attached through nitrogen and mono, di, or trisubstituted with K, C₁₋₆ alkyl-T,

wherein T is selected from the group consisting of formula (I) and formula (II),



Formula I



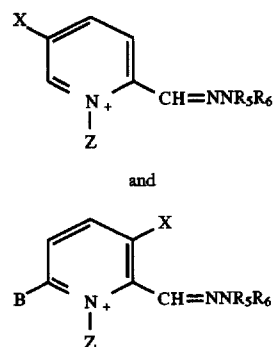
Formula II

wherein R', R'', and R''' are selected from the group consisting of H, C₁₋₆ alkyl, C₁₋₆ fluoroalkyl, C₁₋₆ alkyl with an attached phenyl group, C₁₋₆ alkyl with an attached phenyl group mono, di, or trisubstituted with K, C₁₋₆ fluoroalkyl with an attached phenyl group mono, di, or trisubstituted with K,

wherein K is selected from the group consisting of halogen, COOH, OH, CN, NO₂, NH₂, C₁₋₆ alkyl, C₁₋₆ alkoxy, C₁₋₆ alkylamine, C₁₋₆ alkyl—O—CO—, C₁₋₆ alkyl—O—CO—NH—, C₁₋₆ alkyl—NH—CO—NH—, C₁₋₆ alkyl—NH—CO—O—, C₁₋₆ alkyl NH—CO—, (C₁₋₆ alkyl)₂N—CO—,

Alternatively the novel quaternary pyridinium compounds are represented by the following structural formulas:

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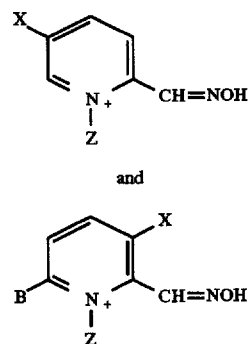


with any counterion to make pharmaceutically acceptable salts, wherein

R₅ is selected from the group consisting of C₁₋₆ alkyl, phenyl, C₁₋₆ alkyl with an attached phenyl group, C₁₋₆ alkyl with an attached phenyl group mono, di, or trisubstituted with K, C₁₋₆ alkyl with an attached naphthyl group, C₁₋₆ alkyl with an attached naphthyl group mono, di, or trisubstituted with K,

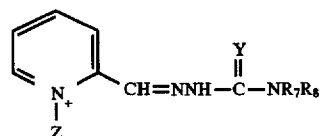
wherein R₆ is selected from the group consisting of H, C₁₋₆ alkyl, phenyl, C₁₋₆ alkyl with an attached phenyl group, C₁₋₆ alkyl with an attached phenyl group mono, di, or trisubstituted with K.

Alternatively the novel quaternary pyridinium compounds are represented by the following structural formulas:



with any counterion to make pharmaceutically acceptable salts.

Alternatively the novel quaternary pyridinium compounds are represented by the following structural formula:



with any counterion to make pharmaceutically acceptable salts, wherein

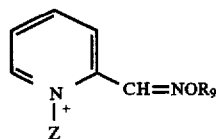
R₇ is selected from the group consisting of C₁₋₆ alkyl, phenyl, C₁₋₆ alkyl with an attached phenyl group, C₁₋₆ alkyl with an attached phenyl group mono, di, or trisubstituted with K, C₁₋₆ alkyl with an attached naphthyl group, C₁₋₆ alkyl with an attached naphthyl group mono, di, or trisubstituted with K, —CO—C₁₋₆ alkyl, —CO—C₁₋₆ alkyl with an attached phenyl group mono, di, or trisubstituted with K,

wherein R₈ is selected from the group consisting of H, C₁₋₆ alkyl, phenyl, C₁₋₆ alkyl with an attached phenyl

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group, C₁₋₆ alkyl with an attached phenyl group mono, di, or trisubstituted with K.

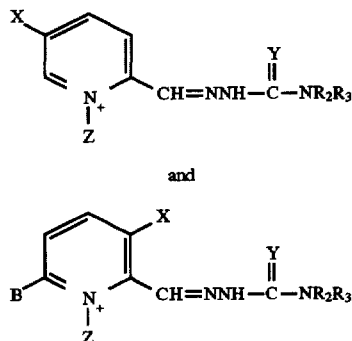
Alternatively the novel quaternary pyridinium compounds are represented by the following structural formula:



with any counterion to make pharmaceutically acceptable salts, wherein

R₉ is selected from the group consisting of C₁₋₆ alkyl with an attached phenyl group, C₁₋₆ alkyl with two attached phenyl groups, C₁₋₆ alkyl with an attached phenyl group mono, di, or trisubstituted with K, C₁₋₆ alkyl with two attached phenyl groups mono, di, or trisubstituted with K, C₁₋₆ alkyl with an attached naphthyl group, C₁₋₆ alkyl with an attached naphthyl group mono, di, or trisubstituted with K.

Alternatively the novel quaternary pyridinium compounds are represented by the following structural formulas:



and any counterion to make pharmaceutically acceptable salts, wherein

Z is selected from the group consisting of C₁₋₆ alkyl, C₁₋₆ alkyl with an attached phenyl group, C₁₋₆ alkyl with an attached naphthyl group, C₁₋₆ alkyl with two attached phenyl groups, C₁₋₆ alkyl with an attached phenyl group mono, di, or trisubstituted with J, C₁₋₆ alkyl with two attached phenyl groups mono, di, or trisubstituted with J, C₁₋₆ alkyl with an attached naphthyl group mono, di, or trisubstituted with J.

wherein J is selected from the group consisting of halogen, COOH, OH, CN, NO₂, NH₂, C₁₋₆ alkyl, C₁₋₆ alkoxy, C₁₋₆ alkylamine, C₁₋₆ alkyl-O-CO-, C₁₋₆ alkyl-O-CO-NH-, C₁₋₆ alkyl-NH-CO-NH-, C₁₋₆ alkyl-NH-CO-O-, C₁₋₆ alkyl-NH-CO-, (C₁₋₆ alkyl)₂N-CO-.

wherein X is selected from the group consisting of OH, C₁₋₆ alkyl-NH-CO-O-, (C₁₋₆ alkyl)₂N-CO-O-, C₁₋₆ fluoroalkyl-NH-CO-O-, (CH₁₋₆ fluoroalkyl)₂-NH-CO-O-.

Y is O or S,

wherein R₂ is selected from the group consisting of pentafluorophenyl, phenyl monosubstituted with K, phenyl disubstituted with K, phenyl trisubstituted with K, and naphthyl.

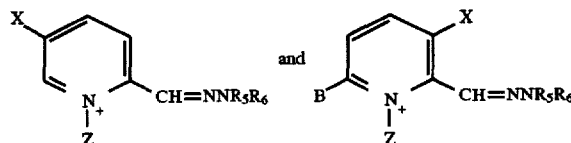
wherein R₃ is selected from the group consisting of H, C₁₋₆ alkyl, phenyl, pentafluorophenyl, phenyl mono, di, or trisubstituted with K, C₁₋₆ alkyl with an attached phenyl group, C₁₋₆ alkyl with an attached phenyl group mono, di, or trisubstituted with K.

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wherein K is selected from the group consisting of halogen, COOH, OH, CN, NO₂, NH₂, C₁₋₆ alkyl, C₁₋₆ fluorinated alkyl, C₁₋₆ alkoxy, C₁₋₆ alkylamine, C₁₋₆ alkyl-O-CO-, C₁₋₆ alkyl-O-CO-NH-, C₁₋₆ alkyl-NH-CO-NH-, C₁₋₆ alkyl-NH-CO-O-, C₁₋₆ alkyl-NH-CO-, (C₁₋₆ alkyl)₂N-CO-.

wherein B is selected from the group consisting of H, C₁₋₆ alkyl.

Alternatively the novel quaternary pyridinium compounds are represented by the following structural formulas:

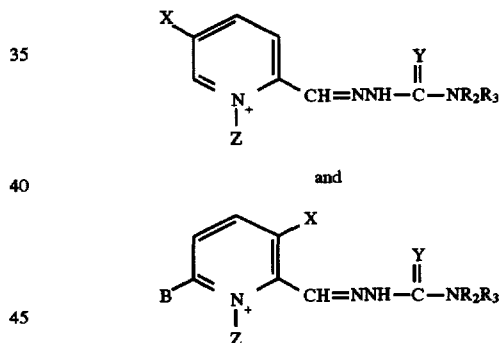


and any counterion to make pharmaceutically acceptable salts, wherein

R₅ is selected from the group consisting of pentafluorophenyl, phenyl monosubstituted with K, phenyl disubstituted with K, phenyl trisubstituted with K, and naphthyl.

wherein R₆ is selected from the group consisting of H, C₁₋₆ alkyl, phenyl, pentafluorophenyl, phenyl mono, di, or trisubstituted with K, C₁₋₆ alkyl with an attached phenyl group, C₁₋₆ alkyl with an attached phenyl group mono, di, or trisubstituted with K.

This invention describes a method of treating mammalian dementia consisting of treating a mammal with a therapeutically effective amount of a compound having the following structure:



and any counterion to make pharmaceutically acceptable salts, wherein

Z is selected from the group consisting of C₁₋₆ alkyl, C₁₋₆ alkyl with an attached phenyl group, C₁₋₆ alkyl with an attached naphthyl group, C₁₋₆ alkyl with two attached phenyl groups, C₁₋₆ alkyl with an attached phenyl group mono, di, or trisubstituted with J, C₁₋₆ alkyl with two attached phenyl groups mono, di, or trisubstituted with J, C₁₋₆ alkyl with an attached naphthyl group mono, di, or trisubstituted with J.

wherein J is selected from the group consisting of halogen, COOH, OH, CN, NO₂, NH₂, C₁₋₆ alkyl, C₁₋₆ alkoxy, C₁₋₆ alkylamine, C₁₋₆ alkyl-O-CO-, C₁₋₆ alkyl-O-CO-NH-, C₁₋₆ alkyl-NH-CO-NH-, C₁₋₆ alkyl-NH-CO-O-, C₁₋₆ alkyl-NH-CO-, (C₁₋₆ alkyl)₂N-CO-.

wherein X is selected from the group consisting of OH, C₁₋₆ alkyl-NH-CO-O-, (C₁₋₆ alkyl)₂N-CO-O-, C₁₋₆ fluoroalkyl-NH-CO-O-, (C₁₋₆ fluoroalkyl)₂-NH-CO-O-.

Y is O or S,

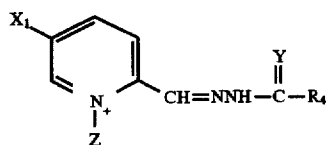
wherein R_2 is selected from the group consisting of H, C_{1-6} alkyl, phenyl, pentafluorophenyl, phenyl mono, di, or trisubstituted with K, C_{1-6} alkyl with an attached phenyl group, C_{1-6} alkyl with an attached phenyl group mono, di, or trisubstituted with K, C_{1-6} alkyl with an attached naphthyl group, naphthyl, C_{1-6} alkyl with an attached naphthyl group mono, di, or trisubstituted with K,

wherein R_3 is selected from the group consisting of H, C_{1-6} alkyl, phenyl, pentafluorophenyl, phenyl mono, di, or trisubstituted with K, C_{1-6} alkyl with an attached phenyl group, C_{1-6} alkyl with an attached phenyl group mono, di, or trisubstituted with K,

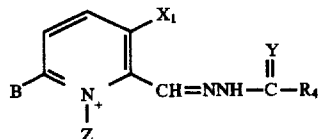
wherein K is selected from the group consisting of halogen, COOH, OH, CN, NO_2 , NH_2 , C_{1-6} alkyl, C_{1-6} fluorinated alkyl, C_{1-6} alkoxy, C_{1-6} alkylamine, C_{1-6} alkyl-O-CO-, C_{1-6} alkyl-O-CO-NH-, C_{1-6} alkyl-NH-CO-NH-, C_{1-6} alkyl-NH-CO-O-, C_{1-6} alkyl-NH-CO-, $(C_{1-6} \text{ alkyl})_2N-CO-$,

wherein B is selected from the group consisting of H, C_{1-6} alkyl,

This invention also describes a method of treating mammalian dementia consisting of treating a mammal with a therapeutically effective amount of a compound having the following structure:



and



and any counterion to make pharmaceutically acceptable salts, wherein

X_1 is selected from the group consisting of H, OH, C_{1-6} alkyl-NH-CO-O-, $(C_{1-6} \text{ alkyl})_2N-CO-O-$, C_{1-6} fluoroalkyl-NH-CO-O-, $(C_{1-6} \text{ fluoroalkyl})_2N-CO-O-$,

Y is O or S,

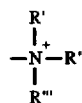
Z is selected from the group consisting of C_{1-6} alkyl, C_{1-6} alkyl with an attached phenyl group, C_{1-6} alkyl with an attached naphthyl group, C_{1-6} alkyl with two attached phenyl groups, C_{1-6} alkyl with an attached phenyl group mono, di, or trisubstituted with J, C_{1-6} alkyl with two attached phenyl groups mono, di, or trisubstituted with J, C_{1-6} alkyl with an attached naphthyl group mono, di, or trisubstituted with J,

wherein J is selected from the group consisting of halogen, COOH, OH, CN, NO_2 , NH_2 , C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} alkylamine, C_{1-6} alkyl-O-CO-, C_{1-6} alkyl-O-CO-NH-, C_{1-6} alkyl-NH-CO-NH-, C_{1-6} alkyl-NH-CO-O-, C_{1-6} alkyl-NH-CO-, $(C_{1-6} \text{ alkyl})_2N-CO-$,

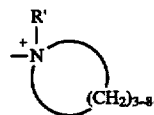
wherein R_4 is selected from the group consisting of C_{1-6} alkyl with a pyridinium ring attached through nitrogen, C_{1-6} alkyl with a pyridinium ring attached through nitrogen and mono, di, or trisubstituted with K, C_{1-6} alkyl with a quinolinium ring attached through nitrogen and mono, di, or trisubstituted with K, C_{1-6} alkyl with an isoquinolinium ring

attached through nitrogen and mono, di, or trisubstituted with K, C_{1-6} alkyl-T,

wherein T is selected from the group consisting of formula (I) and formula (II),



Formula I



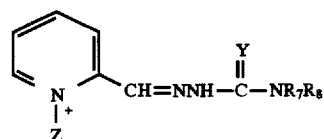
Formula II

wherein R' , R'' , and R''' are selected from the group consisting of H, C_{1-6} alkyl, C_{1-6} fluoroalkyl, C_{1-6} alkyl with an attached phenyl group, C_{1-6} alkyl with an attached phenyl group mono, di, or trisubstituted with K, C_{1-6} fluoroalkyl with an attached phenyl group mono, di, or trisubstituted with K,

wherein K is selected from the group consisting of halogen, COOH, OH, CN, NO_2 , NH_2 , C_{1-6} alkyl, C_{1-6} fluorinated alkyl, C_{1-6} alkoxy, C_{1-6} alkylamine, C_{1-6} alkyl-O-CO-, C_{1-6} alkyl-O-CO-NH-, C_{1-6} alkyl-NH-CO-NH-, C_{1-6} alkyl-NH-CO-O-, C_{1-6} alkyl-NH-CO-, $(C_{1-6} \text{ alkyl})_2N-CO-$,

wherein B is selected from the group consisting of H, C_{1-6} alkyl,

This invention also describes a method of treating mammalian dementia consisting of treating a mammal with a therapeutically effective amount of a compound having the following structure:



and any counterion to make pharmaceutically acceptable salts, wherein

Y is O or S,

Z is selected from the group consisting of C_{1-6} alkyl, C_{1-6} alkyl with an attached phenyl group, C_{1-6} alkyl with an attached naphthyl group, C_{1-6} alkyl with two attached phenyl groups, C_{1-6} alkyl with an attached phenyl group mono, di, or trisubstituted with J, C_{1-6} alkyl with two attached phenyl groups mono, di, or trisubstituted with J, C_{1-6} alkyl with an attached naphthyl group mono, di, or trisubstituted with J,

wherein J is selected from the group consisting of halogen, COOH, OH, CN, NO_2 , NH_2 , C_{1-6} alkyl, C_{1-6} alkoxy, C_{1-6} alkylamine, C_{1-6} alkyl-O-CO-, C_{1-6} alkyl-O-CO-NH-, C_{1-6} alkyl-NH-CO-NH-, C_{1-6} alkyl-NH-CO-O-, C_{1-6} alkyl-NH-CO-, $(C_{1-6} \text{ alkyl})_2N-CO-$,

R_7 is selected from the group consisting of C_{1-6} alkyl, phenyl, pentafluorophenyl, phenyl mono, di, or trisubstituted with K, C_{1-6} alkyl with an attached phenyl group, C_{1-6} alkyl with an attached phenyl group mono, di, or trisubstituted with K, C_{1-6} alkyl with an attached naphthyl group, naphthyl, C_{1-6} alkyl with an attached naphthyl group mono, di, or trisubstituted with K,

wherein R_8 is selected from the group consisting of H, C_{1-6} alkyl, phenyl, pentafluorophenyl, phenyl mono, di, or trisubstituted with K, C_{1-6} alkyl with an attached phenyl

group, C₁₋₆ alkyl with an attached phenyl group mono, di, or trisubstituted with K.

wherein K is selected from the group consisting of halogen, COOH, OH, CN, NO₂, NH₂, C₁₋₆ alkyl, C₁₋₆ fluorinated alkyl, C₁₋₆ alkoxy, C₁₋₆ alkylamine, C₁₋₆ alkyl-O-CO-, C₁₋₆ alkyl-O-CO-NH-, C₁₋₆ alkyl-NH-CO-NH-, C₁₋₆ alkyl-NH-CO-O-, C₁₋₆ alkyl-NH-CO-, (C₁₋₆ alkyl)₂N-CO-.

The following compounds are representative of the invention:

O-Benzyl-N-(1-methyl-2-pyridinemethylene) hydroxylamine chloride (1a).
 O-(Diphenylmethyl)-N-(1-methyl-2-pyridinemethylene) hydroxylamine chloride.
 O-(p-Chlorobenzyl)-N-(1-methyl-2-pyridinemethylene) hydroxylamine chloride.
 O-(p-Methoxybenzyl)-N-(1-methyl-2-pyridinemethylene) hydroxylamine chloride.
 O-(1-Naphtylmethyl)-N-(1-methyl-2-pyridinemethylene) hydroxylamine chloride.
 O-(2-Methyl-1-naphtylmethyl)-N-(1-methyl-2-pyridinemethylene) hydroxylamine chloride.
 O-(2,3,4-Trimethoxybenzyl)-N-(1-methyl-2-pyridinemethylene) hydroxylamine chloride.
 O-(3,3-Diphenylpropyl)-N-(1-methyl-2-pyridinemethylene) hydroxylamine chloride.
 O-Benzyl-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride (1b).
 O-(1-Naphtylmethyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride.
 O-(2-Methyl-1-naphtylmethyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride.
 O-(2,3,4-Trimethoxybenzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride.
 O-(3,3-Diphenylpropyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride.
 O-(1-Naphtylmethyl)-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydroxylamine chloride.
 O-(2-Methyl-1-naphtylmethyl)-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydroxylamine chloride.
 O-Methyl-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride (1e).
 O-Methyl-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydroxylamine chloride.
 O-[2-(1-Methyl-1-piperidinium)ethyl]-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine dichloride.
 O-[2-(Trimethylammonium)ethyl]-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine dichloride.
 O-(p-Chlorobenzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride (1d).
 O-(p-Cyanobenzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride.
 O-(2-Hydroxy-4-nitrobenzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride.
 O-(p-Carboxybenzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride.
 O-(p-Chlorobenzyl)-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydroxylamine chloride.
 O-(p-Cyanobenzyl)-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydroxylamine chloride.
 O-(2-Hydroxy-4-nitrobenzyl)-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydroxylamine chloride.
 O-(p-Methoxybenzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride (1e).
 O-(p-Dimethylaminocarbonyloxy benzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride.
 O-(p-Propylaminocarbonyl benzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride.

O-(p-Ethoxycarbonyl benzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride.
 O-(p-Methoxybenzyl)-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydroxylamine chloride.
 O-(p-Dimethylaminocarbonyloxy benzyl)-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydroxylamine chloride.
 O-(p-Propylaminocarbonyl benzyl)-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydroxylamine chloride.
 O-Benzyl-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene]hydroxylamine chloride (2a).
 O-[2-(1-Methyl-1-piperidinium)ethyl]-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene]hydroxylamine dichloride.
 O-[2-(Trimethylammonium)ethyl]-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene]hydroxylamine dichloride.
 O-Benzyl-N-[3-(N',N'-diethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene]hydroxylamine chloride.
 O-Benzyl-N-[3-(N',N'-difluoroethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene]hydroxylamine chloride.
 O-Diphenylmethyl-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine iodide.
 O-Benzyl-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1,6-dimethyl-2-pyridinemethylene]hydroxylamine chloride.
 O-Benzyl-N-[3-(N',N'-diethylcarbamoyl)hydroxy-1,6-dimethyl-2-pyridinemethylene]hydroxylamine chloride.
 O-Benzyl-N-[3-(N',N'-difluoroethylcarbamoyl)hydroxy-1,6-dimethyl-2-pyridinemethylene]hydroxylamine chloride.
 O-(Diphenylmethyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene]hydroxylamine chloride (2b).
 O-(Diphenylmethyl)-N-[3-(N'-isopropylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene]hydroxylamine chloride.
 O-(Diphenylmethyl)-N-[3-(N'-(2-fluoroethyl)carbamoyl)hydroxy-1-methyl-2-pyridinemethylene]hydroxylamine chloride.
 O-(Diphenylmethyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1,6-dimethyl-2-pyridinemethylene]hydroxylamine chloride.
 O-(Diphenylmethyl)-N-[1,6-dimethyl-3-(N'-isopropylcarbamoyl)hydroxy-2-pyridinemethylene]hydroxylamine chloride.
 O-(Diphenylmethyl)-N-[3-(N'-(2-fluoroethyl)carbamoyl)hydroxy-1,6-dimethyl-2-pyridinemethylene]hydroxylamine chloride.
 O-(p-Methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene]hydroxylamine chloride (2c).
 O-(p-Methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-ethyl-2-pyridinemethylene]hydroxylamine chloride.
 O-(p-Methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-benzyl-2-pyridinemethylene]hydroxylamine bromide.
 O-(p-Methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1,6-dimethyl-2-pyridinemethylene]hydroxylamine chloride.
 O-(p-Methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-ethyl-6-methyl-2-pyridinemethylene]hydroxylamine chloride.
 O-(p-Methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-benzyl-6-methyl-2-pyridinemethylene]hydroxylamine bromide.

O-(p-Methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-(1-naphthylmethyl)-2-pyridinemethylene]hydroxylamine chloride.
 O-(p-Methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-(2-methyl-1-naphthylmethyl)-2-pyridinemethylene]hydroxylamine chloride. 5
 O-(p-Methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-diphenylmethyl-2-pyridinemethylene]hydroxylamine chloride.
 O-(p-Methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-(2-hydroxy-4-nitrobenzyl)-2-pyridinemethylene]hydroxylamine chloride. 10
 O-(p-Methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-(p-carboxybenzyl)-2-pyridinemethylene]hydroxylamine chloride.
 O-(p-Methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-(p-cyanobenzyl)-2-pyridinemethylene]hydroxylamine chloride. 15
 N-[5-(N',N'-Dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene]hydroxylamine chloride (2d).
 N-[3-(N',N'-Dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene]hydroxylamine chloride. 20
 N-[3-(N',N'-Dimethylcarbamoyl)hydroxy-1,6-dimethyl-2-pyridinemethylene]hydroxylamine chloride.
 O-(1-Naphthylmethyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene)hydroxylamine chloride.
 O-(2-Methyl-1-naphthylmethyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene)hydroxylamine chloride.
 O-(2,3,4-Trimethoxybenzyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene)hydroxylamine chloride.
 O-(3,3-Diphenylpropyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene)hydroxylamine chloride. 30
 O-Methyl-N-(5-hydroxy-1-methyl-2-pyridinemethylene)hydroxylamine chloride.
 O-(p-Chlorobenzyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene)hydroxylamine chloride.
 O-(p-Cyanobenzyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene)hydroxylamine chloride.
 O-(2-Hydroxy-4-nitrobenzyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene)hydroxylamine chloride.
 O-(p-Carboxybenzyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene)hydroxylamine chloride. 40
 O-(p-Methoxybenzyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene)hydroxylamine chloride.
 O-(p-Dimethylaminocarbonyloxybenzyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene)hydroxylamine chloride.
 O-(p-Propylaminocarbonylbenzyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene)hydroxylamine chloride.
 O-(p-Ethoxycarbonylbenzyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene)hydroxylamine chloride.
 O-Benzyl-N-[5-(N',N'-dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene]hydroxylamine chloride. 50
 O-Benzyl-N-[5-(N',N'-diethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene]hydroxylamine chloride.
 O-Benzyl-N-[5-(N',N'-difluoroethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene]hydroxylamine chloride.
 O-(Diphenylmethyl)-N-[5-(N'-isopropylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene]hydroxylamine chloride.
 O-(Diphenylmethyl)-N-[5-(N'-(2-fluoroethyl)carbamoyl)hydroxy-1-methyl-2-pyridinemethylene]hydroxylamine chloride. 60
 O-(p-Methoxybenzyl)-N-[5-(N',N'-dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene]hydroxylamine chloride.
 O-(p-Methoxybenzyl)-N-[5-(N',N'-dimethylcarbamoyl)hydroxy-1-ethyl-2-pyridinemethylene]hydroxylamine chloride. 65

O-(p-Methoxybenzyl)-N-[5-(N',N'-dimethylcarbamoyl)hydroxy-1-benzyl-2-pyridinemethylene]hydroxylamine bromide.
 O-(p-Methoxybenzyl)-N-[5-(N',N'-dimethylcarbamoyl)hydroxy-1-(1-naphthylmethyl)-2-pyridinemethylene]hydroxylamine chloride.
 O-(p-Methoxybenzyl)-N-[5-(N',N'-dimethylcarbamoyl)hydroxy-1-(2-methyl-1-naphthylmethyl)-2-pyridinemethylene]hydroxylamine chloride.
 O-(p-Methoxybenzyl)-N-[5-(N',N'-dimethylcarbamoyl)hydroxy-1-diphenylmethyl-2-pyridinemethylene]hydroxylamine chloride.
 O-(p-Methoxybenzyl)-N-[5-(N',N'-dimethylcarbamoyl)hydroxy-1-(2-hydroxy-4-nitrobenzyl)-2-pyridinemethylene]hydroxylamine chloride.
 O-(p-Methoxybenzyl)-N-[5-(N',N'-dimethylcarbamoyl)hydroxy-1-(p-carboxybenzyl)-2-pyridinemethylene]hydroxylamine chloride.
 O-(p-Methoxybenzyl)-N-[5-(N',N'-dimethylcarbamoyl)hydroxy-1-(p-cyanobenzyl)-2-pyridinemethylene]hydroxylamine chloride. 20
 O-Benzyl-N-(1-benzyl-3-hydroxy-2-pyridinemethylene)hydroxylamine bromide.
 O-Benzyl-N-(1-benzyl-5-hydroxy-2-pyridinemethylene)hydroxylamine bromide.
 2-[[[(Aminocarbonyl)hydrazono]methyl]-3-hydroxy-1-methylpyridinium chloride (3a). 25
 2-[[[(Aminocarbonyl)hydrazono]methyl]-5-hydroxy-1-methylpyridinium chloride.
 2[[[(Aminothiocabonyl)hydrazono]methyl]-3-hydroxy-1-methylpyridinium chloride.
 2-[[[(Aminothiocabonyl)hydrazono]methyl]-5-hydroxy-1-methylpyridinium chloride.
 2-Hydrazonomethyl-3-hydroxy-1-methylpyridinium chloride. 30
 2-Hydrazonomethyl-3-hydroxy-1,6-dimethylpyridinium chloride.
 2-Hydrazonomethyl-5-hydroxy-1-methylpyridinium chloride.
 1-Methyl-2-(3-hydroxy-1-methyl-2-pyridinemethylene)hydrazine chloride.
 1-Methyl-1-phenyl-2-(3-hydroxy-1-methyl-2-pyridinemethylene)hydrazine chloride.
 1,1-Diphenyl-2-(3-hydroxy-1-methyl-2-pyridinemethylene)hydrazine chloride.
 1-(2,4-Dinitrophenyl)-2-(3-hydroxy-1-methyl-2-pyridinemethylene)hydrazine chloride. 40
 1-Naphthyl-2-(3-hydroxy-1-methyl-2-pyridinemethylene)hydrazine chloride.
 1-Methyl-2-(5-hydroxy-1-methyl-2-pyridinemethylene)hydrazine chloride.
 1-Methyl-1-phenyl-2-(5-hydroxy-1-methyl-2-pyridinemethylene)hydrazine chloride. I. 1-Diphenyl-2-(5-hydroxy-1-methyl-2-pyridinemethylene)hydrazine chloride.
 1-(2,4-Dinitrophenyl)-2-(5-hydroxy-1-methyl-2-pyridinemethylene)hydrazine chloride. 50
 1-Naphthyl-2-(5-hydroxy-1-methyl-2-pyridinemethylene)hydrazine chloride.
 1-Methyl-2-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene)hydrazine chloride.
 1,1-Diphenyl-2-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene)hydrazine chloride.
 3-Hydroxy-1-methyl-2-[[[(N-phenylaminocarbonyl)hydrazono]methyl]pyridinium chloride (3b).
 1,6-Dimethyl-3-hydroxy-2-[[[(N-phenylaminocarbonyl)hydrazono]methyl]pyridinium chloride. 65

- 5-Hydroxy-1-methyl-2-[[N-phenylaminocarbonyl]hydrazonolmethyl]pyridinium chloride.
- 3-Hydroxy-1-methyl-2-[[N-phenylaminothiocarbonyl]hydrazono]methyl]pyridinium chloride.
- 2-[[N,N-Diphenylaminocarbonyl]hydrazono]methyl]-3-hydroxy-1-methyl pyridinium chloride.
- 1.6-Dimethyl-3-hydroxy-2-[[N-phenylaminothiocarbonyl]hydrazonolmethyl]pyridinium chloride.
- 1.6-Dimethyl-2-[[N,N-diphenylaminocarbonyl]hydrazono]methyl]-3-hydroxy pyridinium chloride.
- 2-[[N,N-Dimethylaminothiocarbonyl]hydrazono]methyl] 3-hydroxy-1-methyl pyridinium chloride.
- 2-[[N-Ethylaminothiocarbonyl]hydrazono]methyl]-3-hydroxy-1-methyl pyridinium chloride.
- 3-Hydroxy-1-methyl-2-[[N-naphthylaminocarbonyl]hydrazono]methyl]pyridinium chloride.
- 2-[[N-(1-Chloro-2-naphthyl)amino carbonyl]hydrazono]methyl]-3-hydroxy-1-methyl pyridinium chloride.
- 5-Hydroxy-1-methyl-2-[[N-phenylaminothiocarbonyl]hydrazonolmethyl]pyridinium chloride.
- 2-[[N,N-Diphenylaminocarbonyl]hydrazono]methyl]-5-hydroxy-1-methyl pyridinium chloride.
- 2-[[N,N-Dimethylaminothiocarbonyl]hydrazono]methyl]-5-hydroxy-1-methyl pyridinium chloride.
- 2-[[N-Ethylaminothiocarbonyl]hydrazono]methyl]-5-hydroxy-1-methyl pyridinium chloride.
- 5-Hydroxy-1-methyl-2-[[N-naphthylaminocarbonyl]hydrazono]methyl]pyridinium chloride.
- 2-[[N-(1-Chloro-2-naphthyl)amino carbonyl]hydrazono]methyl]-5-hydroxy-1-methyl pyridinium chloride.
- 1-Methyl-2-[[N-phenylaminocarbonyl]hydrazono]methyl] pyridinium chloride (3d).
- 2-[[Aminocarbonyl]hydrazono]methyl]-3-(N,N-dimethylcarbamoyl)hydroxy-1-methyl pyridinium chloride (4a).
- 2-[[Aminocarbonyl]hydrazono]methyl]-3-(N,N-dimethylcarbamoyl)hydroxy-1.6-dimethyl pyridinium chloride.
- 2-[[Aminocarbonyl]hydrazono]methyl]-5-(N,N-dimethylcarbamoyl)hydroxy-1-methyl pyridinium chloride.
- 3-(N,N-Dimethylcarbamoyl)hydroxy-1-methyl-2-[[N-phenylaminocarbonyl]hydrazono]methyl]pyridinium chloride (4b).
- 5-(N,N-Dimethylcarbamoyl)hydroxy-1-methyl-2-[[N-phenylaminocarbonyl]hydrazono]methyl]pyridinium chloride.
- 2-(3-Hydroxy-1-methyl-2-pyridinemethylene)-1-[2-(1-pyridinium)acetyl]hydrazine dichloride (5a).
- 2-(1.6-Dimethyl-3-hydroxy-2-pyridinemethylene)-1-[2-(1-pyridinium)acetyl]hydrazine dichloride.
- 2-(5-Hydroxy-1-methyl-2-pyridinemethylene)-1-[2-(1-pyridinium)acetyl]hydrazine dichloride.
- 2-(3-Hydroxy-1-methyl-2-pyridinemethylene)-1-[2-(trimethylammonium)acetyl]hydrazine dichloride.
- 1-[2-(4-Chloro-1-pyridinium)acetyl]-2-(3-hydroxy-1-methyl-2-pyridinemethylene) hydrazine dichloride.
- 2-(1.6-Dimethyl-3-hydroxy-2-pyridinemethylene)-1-[2-(trimethylammonium)acetyl]hydrazine dichloride.
- 2-(1.6-Dimethyl-3-hydroxy-2-pyridinemethylene)-1-[2-(4-chloro-1-pyridine)acetyl]hydrazine dichloride.
- 1-[2-(3-Carboxy-1-isoquinolinium)acetyl]-2-(3-hydroxy-1-methyl-2-pyridinemethylene) hydrazine dichloride.
- 1-[2-(2-Carboxy-4-methoxy-1-quinolinium)acetyl]-2-(3-hydroxy-1-methyl-2-pyridinemethylene) hydrazine dichloride.
- 2-(3-Hydroxy-1-methyl-2-pyridinemethylene)-1-[2-(3-hydroxy-1-pyridinium)acetyl]hydrazine dichloride.

- 2-(3-Hydroxy-1-methyl-2-pyridinemethylene)-1-[2-(1-methyl-piperidinium)acetyl]hydrazine dichloride.
- 2-(5-Hydroxy-1-methyl-2-pyridinemethylene)-1-[2-(trimethylammonium)acetyl]hydrazine dichloride.
- 1-[2-(4-Chloro-1-pyridinium)acetyl]-2-(5-hydroxy-1-methyl-2-pyridinemethylene) hydrazine dichloride.
- 1-[2-(3-Carboxy-1-isoquinolinium)acetyl]-2-(5-hydroxy-1-methyl-2-pyridinemethylene) hydrazine dichloride.
- 1-[2-(2-Carboxy-4-methoxy-1-quinolinium)acetyl]-2-(5-hydroxy-1-methyl-2-pyridinemethylene) hydrazine dichloride.
- 2-(5-Hydroxy-1-methyl-2-pyridinemethylene)-1-[2-(3-hydroxy-1-pyridinium)acetyl]hydrazine dichloride.
- 2-(5-Hydroxy-1-methyl-2-pyridinemethylene)-1-[2-(1-methyl-1-piperidinium)acetyl]hydrazine dichloride.
- 2-(1-Methyl-2-pyridinemethylene)-1-[2-(1-pyridinium)acetyl]hydrazine dichloride (5b).
- O-Benzyl-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydroxylamine chloride.
- 1-(1-Methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(2,6-dichlorophenyl) diazinecarboxamide Iodide.
- 1-(1-Methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(4-methoxyphenyl) diazinecarboxamide Iodide.
- 1-(1-Methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-2-(2,4,6-trichlorophenyl) diazane Iodide.
- 1-(1-Methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(4-chlorophenyl) diazane carboxamide Iodide.
- 1-(1-Methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(2,6-dimethylphenyl) diazane carboxamide Iodide.
- 1-(1-Methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(2,6-difluorophenyl) diazinecarboxamide Iodide.
- 1-(1-Methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(2-ethoxyphenyl) diazane carboxamide Iodide.
- 1-(1-Methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-2-(4-fluorophenyl) diazane Iodide.
- 1-(1-Methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(3-nitrophenyl) diazane carboxamide Iodide.
- 1-(1-Methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(2,6-dichlorophenyl) diazinecarboxamide Iodide.
- 1-(1-Methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(4-bromophenyl) diazane carboxamide Iodide.

It has been found that the novel pyridinium compounds of this invention are good acetylcholinesterase inhibitors in vitro as shown in Tables I and II. The pyridinium compounds also have good activity in vivo as prophylactics and antidotes against nerve agent poisoning as shown in Tables III-VI.

Competitive Inhibition of Acetylcholinesterase. The reversible inhibition studies on electric eel and human erythrocyte AChE were performed at $25^{\circ}\pm 1^{\circ}$ C. in pH 7.6, 0.1M phosphate buffer containing 4% ethanol using S-acetyl thiocholine as a substrate (Ellman, G. L., Courtney, D., Andres, V., and Featherstone, R. M., *Biochem. Pharmacol.* 1961, 7, 88). For analysis of AChE inhibitory potency, electric eel AChE was dissolved in buffer. Human erythrocyte AChE was dissolved in a 0.01M borate buffer (pH 10.2) containing 0.01% protease free bovine serum albumin. 5,5'-

Dithiobis(2-nitrobenzoic acid) (DTNB) and S-acetylthiocholine were prepared in phosphate buffer. All inhibitor solutions were also dissolved in phosphate buffer; some required the addition of 95% ethanol. Increasing absorbance during substrate hydrolysis was monitored on a Varian DMS-90 spectrometer. Duplicate studies were performed on separate days using fresh solutions. No time dependent inhibition was observed over a period of at least one hour. Using three different substrate concentrations and four inhibitor concentrations, a Dixon plot (1/activity vs. inhibitor conc.) was made and the K_i values determined. The K_i value is the dissociation constant of the enzyme-inhibitor complex. Inhibitors with lower K_i values are more potent. In the case of poor inhibitors an IC_{50} was determined by plotting AChE activity vs inhibitor concentration. Only values between 10% and 90% inhibition were used. The exact procedure is as follows.

First, 20 μ L of S-acetylthiocholine (0.075M) and 100 μ L of DTNB (0.10M) were mixed together with phosphate buffer. Inhibitor was then added and the solution mixed well. Next, 50 μ L of electric eel AChE (1.5 units/mL) was added and the solution mixed again. With human enzyme, 20 μ L of a 2 units/mL stock solution was added. Increasing absorbance was monitored at 412 nm. Controls were used for each run in the case of inhibitors that absorb highly at 412 nm. Hydrolysis rates of all inhibitors were negligible. Total volume and organic solvent, if any, was kept constant.

Aldoximes (1a,b,d,e) showed pure competitive inhibition of AChE whereas the semicarbazones (3a,b, 4b) and acyl hydrazones (5a, 5b) showed mixed-type inhibition of AChE (linear, noncompetitive). Results are summarized in Table I.

The 3-hydroxy substituted aldoximes did not inhibit either enzyme well, but did show some selectivity for the electric eel enzyme. The 3-hydroxy derivative of 2-PAM was also tested and did not inhibit either enzyme at concentrations up to 1 mM. Addition of a benzyl group to the aldoxime of 3-hydroxy-2-PAM (1b) improves binding and 1b has an IC_{50} of 500 μ M with electric eel AChE. The methylaldoxime 1c did not inhibit either enzyme at concentrations up to 1 mM. Without the 3-hydroxy substituent, tighter binding occurs and K_i values of 40 μ M and 100 μ M were observed with eel and human enzymes respectively for the benzyl aldoxime 1a.

The bis-pyridinium acyl hydrazones 5a-b were the tightest binding reversible inhibitors with 5a having K_i values of 10 and 20 μ M with electric eel and human erythrocyte AChE, respectively. However, 3-hydroxy substitution did not have the same effect on acyl hydrazones as with the aldoximes. In this case a 3-hydroxy group improved the binding to AChE. The complex kinetics of 5b indicate at least two different binding modes for this inhibitor. At concentrations up to 75 μ M, 5b acts as a noncompetitive inhibitor. However, at higher concentrations, 5b is an uncompetitive inhibitor. Only noncompetitive kinetics were observed for 5a.

All of the semicarbazones (3a,b, 4b) also exhibited non-competitive kinetics. In addition, multiple binding was observed and compound 3b had two distinct K_i values of 30 μ M and 340 μ M with electric eel AChE. Addition of a phenyl group to give the semicarbazone 3b resulted in a decrease of the IC_{50} to 240 μ M with the human enzyme. From the kinetic results, it appears that binding in a hydrophobic pocket near the active site is the most important interaction for the semicarbazones. Compound 4b is the most indicative of this trend. Unlike all other carbamates, it is not a time dependent inhibitor indicating that its position near the active site serine is not equivalent to that occupied

by the aldoximes 2a-e in the E.I complex. Also, the 3-hydroxyl group does not negatively influence binding as with the 3-hydroxy aldoximes which further indicates that binding of the semicarbazones is different from the aldoximes.

Inhibitory data with acetylcholinesterase with halogen substituted semicarbazones and hydrazones are shown in the following table.

TABLE

Kinetic Constants for the Reversible Inhibition of Electric Eel Acetylcholinesterase.

Inhibitor Concentration (mM)	% Inhibition
(1-methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-2-(2,4,6-trichlorophenyl)diazane iodide	
0.45	82
0.35	75
0.15	48
(1-methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(2,6-dichlorophenyl)diazanecarboxamide iodide	
0.32	49

Irreversible Inhibition of Acetylcholinesterase. The irreversible inhibition studies of electric eel and human erythrocyte AChE were performed at $25 \pm 1^\circ$ C. in 0.1M phosphate buffer. Inhibitor and enzyme were incubated in a buffer solution of 1.0 mL total volume and 100 μ L aliquots were taken at 1 min intervals. The aliquots were added to freshly prepared assay solutions containing 20 μ L of S-acetylthiocholine, 100 μ L of DTNB, and 2280 μ L of buffer. Increasing absorbance was monitored at 412 nm. Pseudo first order inhibition constants were determined by measuring the enzymatic activity of acetylcholinesterase after incubating the enzyme with inhibitor for various time periods. Second order inhibition rate constants $k_{obs}/[I]$ are reported in Table II. Inhibitors with higher $k_{obs}/[I]$ values are more potent. Half-lives for inactivation ranged from 10.8 min for 4a to about 9 sec for 2c. The $k_{obs}/[I]$ for 2c was estimated at $>110,000 M^{-1} S^{-1}$ because its rapid inactivation of AChE made it difficult to accurately determine its inhibitory potency. Compounds 2a-c are novel analogs of 2-PAM that also incorporate a dimethylcarbamyl substituent that reacts covalently with AChE. Compounds 2a and 2c have greater anti AChE activity in vitro with human AChE than either pyridostigmine or physostigmine. A notable selectivity for the human enzyme was observed for all of the carbamates. The semicarbazone 4a inactivated AChE very slowly. In contrast, the phenyl substituted semicarbazone carbamate 4b was not a time dependent inhibitor of AChE over a period of 1.5 hours, unlike all other carbamates tested. Both compounds have similar K_i values with electric eel AChE; 4a has a K_i of 100 μ M and 4b has a K_i of 85 μ M. A K_i for the human enzyme could not be determined for 4a because of its rapid inactivation of AChE at inhibitor concentrations exceeding 10 times the enzyme concentration:

Animal Studies—Pretreatment Activity. All animal studies were carried out at Battelle, Inc., in Columbus, Ohio. The results of in vivo mouse evaluations against GD for the compounds tested as intramuscular (i.m.) or oral pretreatment agents are given in Tables III and IV respectively and were obtained as follows. Male ICR mice from Charles River (20 to 30 g average weight) were treated with three different doses of the pyridinium test compound i.m. 15 or 60 min, or by gavage 30 or 120 min before challenge with a dose of $2 \times LD_{50}$ of GD ($LD_{50}=98 \mu$ g/kg without atropine, $LD_{50}=130 \mu$ g/kg with 11.2 mg/kg of atropine). The 24 h LD_{50} of the test compounds administered i.m. or orally were

determined using 5–7 dose groups with 5 animals per dose. As a negative reference treatment, saline was administered instead of the test compound. As a positive control for survival, pyridostigmine (0.1 mg/kg, i.m.; 0.82 mg/kg, orally) was administered to a separate group of animals. All pretreatment groups received atropine sulfate (11.2 mg/kg) and 2-PAM (25 mg/kg) i.m. exactly 10 seconds after GD challenge, using a total dose volume of 0.5 ml/kg body weight. All animals were allocated to pretreatment cells in a randomized block design. Groups of ten mice were used in each experiment and survivors in each group were noted after 24 h. The 24-hour survival of animals pretreated with each dose of the pyridinium test compound was compared with the 24-hour survival observed in the negative reference pretreatment group. A survival difference of at least four is required to identify improved efficacy of the candidate over that observed with the negative reference pretreatment.

Compounds 2a–d, 4a–b, 5a–b were excellent pretreatment agents, affording significant protection against a dose of $2 \times LD_{50}$ of GD. In all cases saline solution was used as the baseline standard (all mice die) and pyridostigmine as the positive control for survival (80–100% of the mice survive).

Carbamate derivatives 2a–2d showed 70 to 90% survival rates at selected doses. After 15 min pretreatment with compound 2a, 2b, or 2d 90% of mice survive a challenge of $2 \times LD_{50}$ of GD at comparable fractions of the LD_{50} 's for these compounds (1/55 LD_{50} of 2a, 1/72 LD_{50} of 2b, 1/67 LD_{50} of 2d). On the other hand, compound 2c afforded a 70% survival rate under the same conditions but at a 1/16,700 fraction of its LD_{50} . Carbamate semicarbazones 4a and 4b and the acyl hydrazones 5a and 5b were also very good i.m. pretreatment agents. Carbamate semicarbazone 4a was exceptionally good as a pretreatment agent. Acyl hydrazones 5a and 5b were also excellent pretreatment agents but their effective doses were much closer to their toxic doses than was the case with 4a. Except for 4a, all remaining pretreatment agents were more effective when given 15 min prior to Soman challenge. The decrease in efficacy at 60 min may be due to transformation of the drugs to inactive metabolites and/or clearance from the animal. It was most noticeable for acyl hydrazones 5a and 5b, which are doubly charged and can be excreted more rapidly. All seven pretreatment agents offered comparable or better protection against GD than the positive control, pyridostigmine.

Carbamate derivatives 2a–2c and carbamate semicarbazone 4b showed excellent activity in the oral pretreatment studies. They afforded 80–100 survival rates at 120 min prior to nerve agent challenge while pyridostigmine alone allowed no survival. In contrast to its good activity in the i.m. studies, bis-pyridinium acyl hydrazone 5a was not good as an oral pretreatment agent. This may be due to fast clearance from the animal requiring higher doses that approach the toxic dose.

Oximes 1a–1f and semicarbazones 3b, 3c and 3d were inactive in the i.m. pretreatment assay. In the case of substituted oxime derivatives a 3-hydroxy substituent significantly reduces drug toxicity (1a>1b). On the other hand increasing the steric bulk of the oximino substituent increases drug toxicity (1b>1c).

Animal Studies—Reactivator Activity. The results of in vivo mouse evaluations against GD and GA for the compounds tested as OP reactivators are given in Tables V and VI and were obtained as follows. Male ICR mice from Charles River (20 to 30 g average weight) were treated with the pyridinium test compound at three different doses administered i.m. 10 seconds after challenge with $2 \times LD_{50}$ of GD or GA (aqueous solution containing 0.9% NaCl). The

test compound was always given simultaneously with atropine sulfate (11.2 mg/kg). As a negative reference treatment group, atropine sulfate (11.2 mg/kg) and 2-PAM (25 mg/kg) were given without the pyridinium test compound (no mice survive). As a positive control for survival, HI-6 (9.6 mg/kg) was administered with atropine sulfate (11.2 mg/kg) to a separate group of animals. All injections were administered i.m. using a dose volume of 0.5 mL/kg body weight. All animals were allocated to treatment cells in a randomized block design. Groups of ten mice were used in each experiment and survivors in each group were noted after 24 h. The 24-hour survival of animals injected with each dose of test compound was compared to the 24-hour survival observed in the negative reference treatment cell. A survival difference of at least four is required to identify improved efficacy of the candidate over that observed with the reference treatment. An alternate procedure used on some compounds was the adjunct efficacy test: the pyridinium test compound was always given simultaneously with atropine sulfate (11.2 mg/kg) and 2-PAM (25 mg/kg). The rest of the procedure is as described above.

Carbamate oxime derivatives 2a, 2b, semicarbazone 3a, carbamate semicarbazone 4b and bis-pyridinium acyl hydrazones 5a and 5b showed significant activity as GD reactivators (Table V). The last two also showed significant activity as GA reactivators (Table VI). 2-PAM was used as the baseline standard while the positive control used HI-6 as the reactivator. Carbamate oximes 2a and 2b afforded 50% and 70% survival rates respectively at a dose 1/16 of their LD_{50} 's. They were also the most toxic of the five derivatives that showed activity as OP reactivators. The greater inhibition of AChE displayed by 2a compared to 2b is matched by its greater toxicity. A 62 fold increase in $k_{obs}/[I]$ accompanied by a 44 fold increase in toxicity indicates that in the case of irreversible inhibitors a compromise must be reached between the degree of inhibition that affords protection from the OP agent and that which prevents the enzyme from performing its physiological role. It is noteworthy that 2a and 2b must be acting as prodrugs of the actual OP reactivator since they do not possess a nucleophile themselves that can displace the OP from the active site of AChE. In the semicarbazone series, a 3-hydroxy substituent increases in vivo activity (3a >> 3c). Semicarbazone 3a afforded 70% survival rate at 1/25 of its LD_{50} when given 10 seconds after a challenge of $2 \times LD_{50}$ of Soman. Semicarbazone 3a was also an excellent reactivator when tested in the adjunct assay: it afforded 90% survival rate when administered along with 2-PAM (25 mg/kg) 10 seconds after Soman challenge. This combination of drugs was more effective than HI-6 in counteracting Soman intoxication. Carbamate semicarbazone 4b afforded 50% survival rate at 1/16 of its LD_{50} when given 10 sec after a challenge of $2 \times LD_{50}$ of Soman. Bis-pyridinium acyl hydrazone 5a was an excellent GD reactivator. It afforded 90% survival rate at a dose of 73 mmol/kg (approx. 1/8 LD_{50}) when given 10 seconds after Soman challenge.

Two hydroxy 2-PAM derivatives (1f, 1g) were prepared and tested for comparison with 2-PAM and with the other substituted oxime derivatives reported in this study. The parent 2-PAM is not an effective reactivator against Soman due to rapid "aging" of the inhibited AChE. Adding a hydroxy substituent to the 3 or 5 position of 2-PAM resulted in no improvement in reactivating ability concurrent with a small increase in toxicity. In addition, these derivatives were not significantly better than 2-PAM in the pretreatment assay. The carbamate of 5-hydroxy-2-PAM chloride, compound 2d, incorporates the structural features of pyridostig-

mine and 2-PAM. It afforded 90% survival rate at a dose $\frac{1}{67}$ of its LD₅₀ when given 15 or 60 minutes prior to Soman challenge.

Summary—Animal Studies. The *in vivo* activity described herein for some of the novel pyridinium compounds of this invention can be summarized as follows: eight compounds (2a–2d, 4a, 4b, 5a, 5b) were effective *i.m.* prophylactics against GD; four compounds (2a–2c, 4b) were effective oral prophylactics against GD; six compounds (2a, 2b, 3a, 4b, 5a, 5b) were good *in vivo* reactivators against GD; two compounds (5a, 5b) were good *in vivo* reactivators against CA; five compounds (2a, 2b, 4b, 5a, 5b) were both excellent prophylactics and good reactivators against GD. Compounds 4a and 3a form a set in which an excellent prophylactic (4a) is transformed by AChE into a good OP reactivator (3a). Structural requirements for prophylactic activity are a carbamate moiety and/or a second quaternary center. Since carbamates may act as prodrugs for the actual reactivators (*i.e.* 3a and 4a), the latter structural requirements also apply for AChE reactivators in addition to semicarbazones having a free OH group in the pyridine ring.

Monkey Cognition/Memory Studies. The cohort of monkeys for this study included the following macaques: 4 aged male rhesus (*macaca mulatta*), 1 young mature male rhesus, 1 aged female rhesus, 2 young mature male pigtailed (*macaca nemestrina*), 3 young mature female pigtailed, and 1 young mature female cynomolgous (*macaca fascicularis*). The young mature animals were 8–15 years old and the aged animals were 21–45 years old. The animals were originally obtained from commercial suppliers or as reproductive culls. All of these animals had participated in previous drug studies involving short-term administration of low doses of agents affecting cognition/memory. They were allowed to wash out any prior drug effects for at least 4 weeks prior to this study. The monkeys were housed in individual stainless steel cages located in the Medical College of Georgia Animal Behavior Center, an A.A.A.L.A.C.-approved facility designed for the care and maintenance of non-human primates. At certain times when animals are not tested routinely (during quarantine after arrival or during washout periods from drug studies) they were allowed access to an enclosed outdoor exercise facility on an individual basis. Both cages and exercise facilities contained perch bars and play objects. The animals were maintained on water (unlimited) and standard laboratory monkey chow supplemented with fruits and vegetables. The animals were maintained on a feeding schedule such that approximately 15% of their normal daily (except weekends) food intake was derived from reinforcement food pellets (commercial composition of standard monkey chow and banana flakes) obtained during experimental sessions. The remainder was made available following each test session. On weekends the animals were fed twice per day. When not participating in testing sessions, the animals were permitted to watch television programs to permit psychological well being. Also, monkeys received human visual contact and attention each day, especially just after testing sessions. These protocols for maintenance and testing of the animals received prior approval by the Institutional Committee on Animal Use for Research and Education.

DMTS (delayed matching to sample) testing took place with the animals in their home cage with the room darkened and the computer and operator isolated from the subjects. Daily sessions consist of 96 trials. Trials begin by illumination of a sample key with one of three colored discs. Monkeys were trained to depress the illuminated sample key and this action terminated illumination of the sample key for

a delay interval lasting 0–120 sec. Following the delay interval, two choice keys (positioned below and to either side of the stimulus key), but not the sample key, were then illuminated. One of the two choice keys was the same color as the sample key had been prior the delay, while the other (incorrect) choice key was one of the two remaining colors. If the monkey matched (*i.e.*, pressed the choice key which was the same color as the sample key had been), that response was rewarded with a 300 mg banana flavored food pellet. A non-corrective procedure was employed, thus incorrect responses were followed by a completely new trial. Following both correct and incorrect responses there was an inter-trial interval of 3 sec. Monkeys exhibit individual capabilities to maintain matching performance following various delay intervals, and the longest delay chosen for a particular monkey is that which consistently allows correct matching at just above chance levels (approximately 60% correct). In general, the length of delay interval is adjusted until four levels of performance difficulty are found: 1) the least difficult 0 delay (mean=85–100% correct); 2) a short delay interval (means ranging from 75–85% correct); 3) a medium delay interval (means ranging from 65–75% correct) and 4) a long delay interval representing each animal's limit in terms of DMTS performance (55–65% correct). Baseline data was obtained following the *i.m.* administration of vehicle (sterile normal saline). Drugs were administered *i.m.* (gastrocnemius muscle) in a volume of 0.3 ml. At 10 min after drug or vehicle injection, testing panels were attached to the front of the animal's home cage and the trials immediately initiated. A minimum drug "washout" period of 2 days was allowed between sessions in which a drug was administered. Generally, vehicle was administered on Monday, drug was administered on Tuesday and Thursday, and on Wednesday and Friday animals were tested, but no drug or vehicle was administered. After drug administration, a return to baseline performance was established in each animal before again administering drug.

Rat Behavioral Studies. Male, outbred Wistar rats weighing 250–300 g were obtained from Harlan, Sprague-Dawley (Indianapolis, Ind.) and housed separately in our animal care facility for 1 week prior to experimentation. At the time of the experiment the 40 animals were randomly assigned to one of 4 treatment groups, a saline vehicle group, or a 50 or 100 or 200 $\mu\text{g/kg}$ 4b group. Vehicle (1 ml/kg body weight) was administered *i.p.* 30 min prior to testing in the Morris Water Maze apparatus. The apparatus consisted of a water (room temperature)—filled tub 1.2 m in diameter. A mounting platform was fixed in place and slightly submerged in the north-west quadrant of the tub. The platform was similar in hue to the inner surface of tub so as to make it difficult to visualize. The tub was always maintained in the same orientation with respect to visual cues placed on the walls, etc., around the testing room. Rats were tested by placing the animal in the water facing away from the platform. Four consecutive trials were administered with 10 min between trials. In each successive trial the rats were placed first in the south quadrant of the tub, followed by the north, east and west quadrants. The time required for the rat to find (place at least 2 paws) the platform was monitored to the nearest 0.1 sec. All rats found the platform in at least 90 sec.

For nicotinic receptor binding assays, rats were decapitated and the brain was removed and dissected over ice. Cerebral cortical tissue was homogenized (teflon-glass) in ice cold 50 mM Tris-HCl buffer containing 120 mM NaCl, 5 mM KCl, 1 mM MgCl₂, and 2 mM CaCl₂ (pH 7.0). The tissue homogenate was centrifuged at 17,000 rpm (37,223 g) for 20 min at 0° C. The pellet was washed twice by

centrifugation and resuspended in fresh buffer. The Bio-Rad protein assay was used to determine the protein concentration of homogenates with methylated bovine albumin as the standard. [^3H]cytisine was used to determine nicotinic receptor binding parameters according to the method described by Pabreza et al., [Pabreza, L. A., Dhawasn S. and Kellar, K. J. [^3H]cytisine binding to nicotinic cholinergic receptors in brain. *Mol. Pharmacol.* 39: 9-12(1991)] with some modification. [^3H]cytisine (2 nM) was incubated with 1 of 10 concentrations from 0.001 to 100 μM nicotine, carbachol or 4b and with 0.525 mg protein in a final volume of 0.25 ml at 2° C. for 75 min; 10 μM (–)nicotine was used to determine nonspecific binding. Separation of bound from free ligand was accomplished by rapid filtration (Brandel Cell Harvester, Gaithersburg, Md.) under reduced pressure (20–25 inch Hg) through glass fiber filter paper and by washing with ice-cold washing buffer (Tris-HCl (50 mM) 4×4 ml). Filter strips were pre-soaked in 0.05% polyethyleneimine for 2 hr. Following the filtration, filters were placed in 7-ml polypropylene scintillation vials and 4.5 ml of Scintillation fluid was added. The radioactivity was measured in a scintillation spectrophotometer. The fractional specific binding of [^3H]cytisine in the presence of unlabeled ligand was analyzed using a nonlinear curve fitting program (Table Curve, Jandel Scientific, Corte Madera, Calif.) which fits the data to either one or two classes of binding sites and assists in determining whether the two-site model is better than the one-site fit of the data. The resulting IC_{50} values represent the mean of 3 separate binding experiments which were performed in duplicate.

Data derived from the more than 2 treatment groups were analyzed using one- or two-way analysis of variance (ANOVA) with repeated measures. In cases where significant differences were found or to compare only 2 treatment groups, Student's t-test was employed to determine which group means differed. Means were considered to be statistically significant at the $p < 0.05$ level. Statistical comparisons for all behavioral studies were made on the baseline data.

In the first series of experiments, 4b was compared with the nicotinic agonists nicotine and carbachol for the ability to displace [^3H]cytisine from binding sites on membranes derived from rat cerebral cortex. The results of the experiment are presented in the following Table. Nicotine specific fractional binding was fit to a one-site model and exhibited the greatest potency. Carbachol also was fit to a one-site model and exhibited about 18 fold less potency than nicotine. In contrast, 4b binding fit well to a two-site model in which the high affinity binding was about 600 fold less potent than nicotine and about 30 fold less potent than carbachol. Thus, 4b appears to possess significant, albeit weak affinity for neuronal nicotinic receptors. In contrast, the related carbamate cholinesterase inhibitor, physostigmine (which is a tertiary amine at physiological pH) exhibited no selective binding to nicotinic receptors, even at mM concentrations (data not shown).

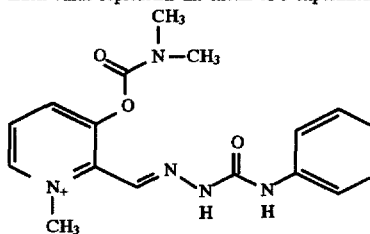
TABLE

Comparison of Comparison of Cortical Nicotinic Receptor Binding Parameters as Measured by [^3H]Cytisine Displacement		
Drug	IC_{50} (μM)	
	High Affinity Site	Low Affinity Site
Nicotine	0.0396	
Carbachol	0.6988	

TABLE-continued

Comparison of Comparison of Cortical Nicotinic Receptor Binding Parameters as Measured by [^3H]Cytisine Displacement		
Drug	IC_{50} (μM)	
	High Affinity Site	Low Affinity Site
4b	23.394	2,630.0

Each value represents the mean of 3 experiments performed in duplicate.



Structure of 3-(N,N-Dimethylcarbamoyl)hydroxy-1-methyl-2-[[N-phenylaminocarbonyl]hydrazono]methylpyridinium chloride (MHP-133, 4b) or 1-(1-methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(phenyl)diazanecarboxamide chloride.

In an initial series of experiments employing 4b in monkeys performing the DMTS task we examined a broad range of doses between 1.25 and 150 $\mu\text{g}/\text{kg}$, i.m. in 5 monkeys in an ascending series. The cohort employed in this series consisted of 4 aged male rhesus and 1 young mature male pigtail. The dose-response relationship for medium and long delay intervals is depicted in FIG. 1. 4b produced a graded increase in performance of the DMTS task, particularly at long delay intervals (0 delay and short delay intervals were not affected) up to 60 $\mu\text{g}/\text{kg}$. A second peak of activity, particularly at medium delay, occurred at 100 $\mu\text{g}/\text{kg}$. However, above 70 $\mu\text{g}/\text{kg}$ 2 animals would not complete their sessions, and above 150 $\mu\text{g}/\text{kg}$, a third animal failed to complete his sessions. When an animal fails to complete a session while on drug regimen it often signifies the onset of some untoward effect which interferes with task performance. The 3 animals who failed to achieve the highest doses were from the aged group, and occasionally aged animals will not complete sessions even in the absence of drugs. We were able to repeat the 100 $\mu\text{g}/\text{kg}$ dose in one of these animals several months later, and this animal had no difficulty completing his sessions, and exhibited no apparent deleterious effect of the dose.

In the second series, an additional 7 animals were tested with an ascending regimen of 3 doses of 4b, 30, 60 and 100 $\mu\text{g}/\text{kg}$, corresponding to the doses which appeared to provide the greatest improvement in the initial study. One of these was the aged male rhesus mentioned above who was in the original study, and who did not receive doses higher than 70 $\mu\text{g}/\text{kg}$. Of these 7 animals, one aged male rhesus was a non-responder, who did not improve performance significantly at any of the 3 doses. The possibility existed that this particular animal may have responded to one or more doses other than the 3 employed, however, this possibility was not explored and the data for this animal was omitted from further analysis. Examination of the individual data indicated that there was no apparent differences between the responsiveness to 4b between animals segregated into gender, species or age groups. However, these individual groups were too small to permit rigorous statistical analysis. Data were combined for performance at 30, 60 and 100 $\mu\text{g}/\text{kg}$.

kg for all 11 animals of the entire study (omitting the 1 non-responder) and the results presented in FIG. 2. The improvement observed to the 3 doses was essentially similar to that observed in the initial study. One difference was that the improvement in performance obtained to 100 µg/kg in the initial study for medium delay intervals was not maintained in the larger group. Also, while the dose exhibiting greatest improvement in the initial study appeared to be the 60 µg/kg dose, in the larger group, improvement appeared to be greatest for the 30 and 100 µg/kg doses. Interestingly, while performance at long delay intervals for the 60 µg/kg dose in the larger group did not improve to statistically significant levels, it appears that some of the improvement was shifted from long delay to medium delay intervals (FIG. 2). Performance was also analyzed during testing on the day following each administration of 4b. There was no significant improvement noted on the day following drug administration for any of the doses and for any of the delay intervals (data not shown). There was also no significant effect produced by 4b on choice latencies (time interval between sample illumination and key press).

For each responder in the study a most effective or "best dose" was selected. For the 7 animals involved in the second phase this involved 1 of the 3 doses employed. For the 5 animals involved in the initial phase, only doses between 30 and 100 µg/kg were considered. Thus, the average increase in DMTS performance at best dose should be considered a conservative estimate since only doses from this restricted range were employed. For the cohort, 5 of the animals' best dose was 30 µg/kg, 1 was 50 µg/kg, 2 was 60 µg/kg, 1 was 70 µg/kg and 2 was 100 µg/kg. Thus, the average best dose was 54±8 µg/kg. The data for average change in DMTS performance for best dose is depicted in FIG. 3. Although there was a statistically significant improvement in overall performance (all delay intervals combined), there was a delay-dependent increase in performance with the greatest and only statistically significant improvement observed for long delay intervals. In fact, at long delay intervals, improvement averaged 18 percentage points above baseline. Expressed as percent of baseline DMTS performance, the improvement for this group after 4b administration averaged 32.2%.

In the final series, 4 groups of young mature rats were tested in the Morris Water Maze apparatus. Rats which received saline (vehicles) prior to testing exhibited a steep learning curve. Performance (platform finding) required about 64 sec on average for the initial trial and this decreased to only about 20 sec by the fourth trial. 4b did not significantly affect the first or second trial latencies, but the 50 and 100 µg/kg doses produced an increase in the slope of the learning curve. This was particularly evident on the third trial.

Animal Studies—Discussion. While the neurochemical basis for the use of cholinesterase inhibitors is generally ascribed to their ability to increase synaptic levels of acetylcholine, this class of compounds when administered on a chronic basis often results in tolerance to their pharmacological, biochemical and toxic actions. Such tolerance occurs in two phases, acute and chronic. The latter phase is usually associated with postsynaptic muscarinic receptor down-regulation. The more acute phase is associated with a decrease in acetylcholine biosynthesis and release which is mediated either through activation of presynaptic muscarinic inhibitory feedback receptors, or through the high intra-terminal concentration of acetylcholine achieved. Interestingly, two recent reports concur in their findings that cholinesterase inhibitors may increase

rather than decrease acetylcholine release from brain tissue derived from Alzheimer's patients (Nilsson, L., Nordberg, A., Hardy, J., Wester, P. and Winblad, B., Physostigmine restores ³H-acetylcholine efflux from Alzheimer brain slices to normal level, *J. Neural Trans.* 67:275-285 (1986); Nilsson, L., Adem, A., Hardy, J., Winblad, B. and Nordberg, A., Do tetrahydroaminoacridine (THA) and physostigmine restore acetylcholine release in Alzheimer brains via nicotinic receptors?, *J. Neural Transm.* 70:357-368 (1987).). It is not clear why the muscarinic inhibitory circuit is not activated under these circumstances, however, it is known that in neuromuscular tissue, presynaptic facilitation by physostigmine (cholinesterase inhibitor) is mediated through presynaptic nicotinic receptors. Also, when the nicotinic system is fully operative, the muscarinic inhibitory system is much less effective. If this were true for central cholinergic neurons, it could explain the apparently inactive status of the muscarinic inhibitory feedback system in Alzheimer's tissue.

One study has indicated that the facilitatory action of cholinesterase inhibitors on acetylcholine release in Alzheimer's Disease tissue is blocked by nicotinic antagonists such as mecamylamine. While these relationships may seem circumstantial, they do help to explain the findings of one of the inventors and those of others that central nicotinic antagonists such as mecamylamine produce significant and severe memory difficulties in animal models (Dills, S. L. and Berry, C. A. Effect of cholinergic drugs on passive avoidance in the mouse. *J. Pharmacol. Exper. Ther.* 158:279-285 (1967); Chiappeta, L. and Jaravik, M. E., Comparison of learning impairment and activity depression produced by two classes of cholinergic blocking agents, *Arch. Int. Pharmacodyn. Therap.* 179: 161-166 (1969); Goldberg, M. E., Sledge, K., Hefner, M. and Robichaud, R. C., Learning impairment after three classes of agents which modify cholinergic function, *Arch. Int. Pharmacodyn.* 193:226-235 (1971); Blozovski, D., Deficits in passive avoidance learning in young rats following mecamylamine injections in the hippocampo-entorhinal area. *Exper. Brain Res.* 50:442-448 (1983)) and in man (Stolerman, I. P., Goldfarb, T., Fink, R. and Jarvik, M. E., Influencing cigarette smoking with nicotine antagonists. *Psychopharmacol.* (Berlin) 28:247-259 (1973)).

These findings underlie the rationale for the present invention, that is, to develop a drug that exhibits both nicotinic activity and AChE inhibition and thus could produce significantly improved memory enhancement with respect to that produced by either nicotine or an AChE inhibitor alone. In fact, it has been known since 1966 that physostigmine can facilitate the pharmacological actions of nicotine (Armitage, A. K., Milton, A. S. and Morrison, c. F., Effects of nicotine and some nicotine-like compounds injected into the cerebral ventricles of the cat, *Br. J. Pharmac.* 27:33-45 (1966)). This has been attributed to the ability of nicotine to cause acetylcholine release and through physostigmine's ability to prevent acetylcholine degradation (Balfour, D. J. K., The effects of nicotine on brain neurotransmitter systems, *Pharmac. Ther.* 16:269-282 (1982)).

The delayed matching-to-sample (DMTS) task allows the measurement of abilities which are relevant to human aging, such as attention, strategy formation, reaction time in complex situations and memory for recent events. A similar version of this task has been employed to demonstrate cognitive impairment in Alzheimer's disease (AD) patients (Irie, E., Kessler, J. and Markowitsch, H. J., Primate learning tasks reveal strong impairments in patients with presenile or senile dementia of the Alzheimer type, *Brain Cogn.* 6:429

(1987)). It is of interest to note that different classes of memory enhancing agents do not all produce selective effects at long delay intervals. The α_2 -adrenergic agonist clonidine improves DMTS performance to the greatest extent at medium delays while the ACHE inhibitor physostigmine acts more evenly across short, medium and long delays (Terry, A. V., Jackson, W. J. and Buccafusco, J. J., Effects of concomitant cholinergic and adrenergic stimulation on learning and memory performance by young and aged monkeys, *Cerebral Cortex* 3:304-312 (1993)). Both clonidine and nicotine improve performance for at least 24 hr after administration, whereas physostigmine evokes responses only on the day of administration.

In the present invention, 4b appears to be more nicotine-like since it appears to affect mainly the long delay intervals. On the other hand, nicotine evokes an enhancement of DMTS performance which is still measurable 24 hr after the injection (Buccafusco, J. J. and Jackson, W. J., Beneficial effects of nicotine administered prior to a delayed matching-to-sample task in young and aged monkeys, *Neurobiol. Aging* 12:233-238 (1991)). The improvement in performance to 4b is relegated to the day of administration. Thus, 4b appears to exhibit characteristics of both physostigmine and nicotine in the DMTS task, which fits well with the hypothesis that the drug may enhance memory function through both ACHE inhibition and nicotinic receptor stimulation.

A second feature of this hypothesis was that combining nicotinic stimulation with ACHE inhibition should result in a synergistic action on memory performance. Dose-response studies employing physostigmine or nicotine alone generally produce improvements of 5-10% points, and generally increases of this magnitude occur for but one dose in the series. In the present invention 4b produced a robust increase in DMTS performance up to 14% points over 2-3 doses. Thus, 4b may exhibit a wider therapeutic response window than either physostigmine or nicotine. The drug improves memory performance in both young and aged monkeys and in young rats tested in the Morris Water Maze. It was interesting that rats and monkeys received mnemonic benefit from similar doses despite the differences in the route of administration and the nature of the task. It is clear, however, that the effect of the drug at long delay intervals in the DMTS task, is more consistent with a selective action of the drug on problems that are most taxing to memory. The drug does not act as a stimulant, increasing lever pressing activity, since choice latencies in the DMTS paradigm were not affected.

Summary. In summary, 4b is a novel ACHE inhibitor which produces dramatic improvement in memory performance, particularly for the most taxing challenges to recall. The drug is centrally-active while retaining a permanent positive charge at physiological pH. Its acetylcholine-like structure may impart some nicotinic activity which adds or perhaps synergizes with its AChE inhibiting activity to produce its mnemonic stimulating action.

Drug Delivery. For the prophylaxis of OP poisoning and for the treatment of OP overdose, Alzheimer's disease (senile dementia), presenile dementia of the Alzheimer's type, Korsakoffs syndrome (psychosis), age-related memory impairment, multi-infarct dementia, Parkinsonian dementia, Down's syndrome, and posttraumatic dementia (dementia pugilistica), Myasthenia Gravis, high blood pressure, glaucoma, and abdominal distension the pyridinium compounds of the present invention may be administered orally, topically or parenterally. The term parenteral as used includes subcutaneous injection, intravenous, intramuscular,

intrasternal injection or infusion techniques. The dosage depends primarily on the specific formulation and on the object of the therapy or prophylaxis. The amount of the individual doses as well as the administration is best determined by individually assessing the particular case.

The pharmaceutical compositions containing the active ingredient may be in a form suitable for oral use, for example as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules or syrups or elixirs. Dosage levels of the order to 0.2 mg to 140 mg per kilogram of body weight per day are useful in the treatment of above-indicated conditions (10 mg to 7 gms per patient per day). The amount of active ingredient that may be combined with carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

For injection, the therapeutic amount of pyridinium compounds of the present invention will normally be in the dosage range from 0.2 mg to 140 mg/kg of body weight. Administration is made by intravenous, intramuscular or subcutaneous injection. Accordingly, pharmaceutical compositions for parenteral administration will contain in a single dosage form about 10 mg to 7 gms of pyridinium compound of the present invention. In addition to the active ingredient, these pharmaceutical compositions will usually contain a buffer, e.g. a phosphate buffer which keeps the pH in the range from 3.5 to 7 and also sodium chloride, mannitol or sorbitol for adjusting the isotonic pressure.

A composition for topical application can be formulated as an aqueous solution, lotion, jelly or an oily solution or suspension. A composition in the form of an aqueous solution is obtained by dissolving the pyridinium compounds of the present invention in aqueous buffer solution of pH 4 to 8.5 and if desired, adding a polymeric binder. An oily formulation for topical application is obtained by suspending the pyridinium compounds of the present invention in an oil, optionally with the addition of a swelling agent such as aluminium stearate and/or a surfactant.

Synthetic Methods. All common chemicals and solvents were reagent grade or better. The purity of each compound was checked by ^1H NMR, mass spectroscopy, thin-layer chromatography (TLC), and elemental analysis. Results are consistent with the proposed structures. Melting points were obtained on a Büchi capillary melting point apparatus and are uncorrected. ^1H NMR spectra were recorded with a Varian Gemini 300 MHz NMR spectrometer; chemical shifts are reported in parts per million (δ) from an internal tetramethylsilane standard. Mass spectra were recorded on a Varian Mat 112s spectrometer. Elemental analyses were performed by Atlantic Microlab in Atlanta, Georgia. 3-Hydroxy-2-pyridine carboxaldehyde (Stempel, A. and Buzzi, E. C., *J. Am. Chem. Soc.* 1949, 71, 2969; Ginsburg, S. and Wilson, I. B., *J. Am. Chem. Soc.* 1957, 79, 481, incorporated herein by reference) was prepared from 3-hydroxy-2-hydroxymethyl pyridine by oxidation using manganese dioxide (Demerseman, P., Kiffer, D., Debussche, L., Lion, C., Royer, R., and S. Roumanou, H., *Eur. J. Med. Chem.* 1988, 23, 63, incorporated herein by reference). Girard's reagent "P" (1-(carboxymethyl)pyridinium chloride), 4-phenylsemicarbazide, and 3-hydroxy-6-methyl-2-pyridinemethanol were purchased from Aldrich Chemical Company.

The oxime, hydrazone, semicarbazone and acyl hydrazone derivatives described herein were prepared using standard methodology. Many different O-substituted hydroxylamines can be prepared by known procedures (Grochowski,

E. and Jurczak, J., *Synthesis* 1976, 682; Schumann, E. L., Heinzelman, R. V., Greig, M. E., and Veldkamp, W., *J. Med. Chem.* 1964, 7, 329, incorporated herein by reference) to afford the various substituted oximes described herein. Several substituted hydrazines are commercially available (i.e. methylhydrazine, 1,1-dimethylhydrazine, 1,1-diphenylhydrazine, from Aldrich Chemical Co.) and others can be synthesized by standard methodology (i.e. Hoffman degradation of ureas, reduction of N-nitroso compounds, reduction of diazonium salts) to afford the hydrazone derivatives described herein. Several substituted semicarbazides are commercially available (i.e. 4-phenyl-3-thiosemicarbazide, 4,4-diphenylsemicarbazide, 4,4-dimethyl-3-thiosemicarbazide, 4-ethyl-3-thiosemicarbazide) and others can be synthesized by standard methodology (reduction of N-nitro ureas). Commercially available Girard's reagent "T" ((carboxymethyl) trimethylammonium chloride hydrazide) can be used instead of Girard's reagent "P" to obtain 2-(trimethylammonium) acetyl hydrazine derivatives similar to 5a and 5b. Other hydrazides for the synthesis of various acyl hydrazones described herein can be made by known methodology (Vogel, A. I., in *A Textbook of Practical Organic Chemistry*; Longman Group Limited: London, 1972; pp.976-978, incorporated herein by reference), which involves the reaction between an amine and ethyl chloroacetate, followed by reaction with hydrazine hydrate. Conversion of the 3 or 5-hydroxy moiety into a carbamate was accomplished using N,N-dimethylcarbamyl chloride. Other N,N-disubstituted carbamates can be made by reaction between commercially available triphosgene and a disubstituted amine, followed by reaction with the 3 or 5-hydroxy pyridine derivative. N-monosubstituted carbamates can be prepared by reaction of the free 3—OH group on the pyridine ring and any of numerous isocyanates that are commercially available. In the case of fluorinated derivatives, the synthesis of the appropriate fluoroalkylamine (some are commercially available) is required, followed by reaction with phosgene to generate the desired isocyanate. Quaternization of the pyridine nitrogen was carried out using methyl iodide in a sealed glass pressure vessel and was followed by anion exchange using a biphasic mixture of silver chloride and the methiodide in acetonitrile/water. Other salts of the pyridinium compounds described herein can be made in similar fashion, by methathesis involving the silver salt of the desired counterion and the methiodide formed in the initial alkylation of the pyridine nitrogen. The usefulness of this procedure has been studied with 2-PAM derivatives (Kondritzer, A. A., Ellin, R. I., and Edberg, L. J., *J. Pharm. Sci.* 1961, 50, 109, incorporated herein by reference).

The following examples are given to illustrate the invention and are not intended to limit it in any manner:

EXAMPLE 1

Preparation of O-Benzyl-N-(1-methyl-2-pyridinemethylene) Hydroxylamine Chloride (1a).

O-Benzyl-N-(2-pyridinemethylene) hydroxylamine. O-Benzylhydroxylamine hydrochloride (12.77 g, 0.08 mol) was added to a solution of NaOH (3.2 g, 0.08 mol) in 80 mL 1:1 EtOH/H₂O. Pyridine-2-carbaldehyde (7.6 mL, 0.08 mol) was added to this solution and the resulting mixture was stirred at room temperature for 10 h. Excess water was removed under vacuum and the residue was fractionally distilled under reduced pressure (14 mm Hg). The product was obtained as a yellow oil in the fraction distilling at 85°-90° C. (11.3 g, 67%). ¹H NMR (Me₂SO-d₆) δ: 5.25 (s, 2H), 7.24 (t, 1H), 7.32-7.44 (m, 5H), 7.66 (t, 1H), 7.78 (d, 1H), 8.23 (s, 1H), 8.58 (d, 1H).

O-Benzyl-N-(1-methyl-2-pyridinemethylene) hydroxylamine iodide. Methyl iodide (14.3 mL, 0.23 mol) was added to a solution of O-Benzyl-N-(2-pyridinemethylene) hydroxylamine (11.3 g, 0.05 mol) in 75 mL of acetonitrile. The resulting mixture was heated in a pressure vessel at 65° C. for 24 h. The solvent was removed in vacuo and the residue was triturated with acetone to afford the product as a light orange solid (17.2 g, 92%), m.p. 125°-7° C. (dec.). ¹H NMR (Me₂SO-d₆) δ: 4.36 (s, 3H), 5.38 (s, 2H), 7.36-7.49 (m, 5H), 8.09 (t, 1H), 8.36 (d, 1H), 8.55 (t, 1H), 8.84 (s, 1H), 9.00 (d, 1H).

O-Benzyl-N-(1-methyl-2-pyridinemethylene) hydroxylamine chloride. Silver chloride (freshly prepared from 14.6 g AgNO₃ and excess conc. HCl) was added to a solution of 16.2 g (0.05 mol) of O-Benzyl-N-(1-methyl-2-pyridinemethylene)hydroxylamine iodide in 800 mL H₂O. The suspension was vigorously stirred with a mechanical stirrer for 4 h at room temperature. The yellow precipitate was filtered off and the filtrate was concentrated to dryness and coevaporated several times with acetone. Traces of solvent are removed by evaporation at 50° C. in a rotary evaporator at 0.8 mm Hg overnight. The yellow solid that remains (11.63 g) is recrystallized from EtOH/acetone with scratching to obtain 8.1 g (68%) of analytically pure product as beige microscopic needles; m.p. 145°-6° C. (dec.). Anal. Calcd. for C₁₄H₁₅ClN₂O x H₂O: C, 61.87; H, 5.93; N, 10.31; Cl, 13.05. Found: C, 61.94; H, 5.94; N, 10.31; Cl, 13.10. ¹H NMR (Me₂SO-d₆) δ: 4.38 (s, 3H), 5.38 (s, 2H), 7.36-7.48 (m, 5H), 8.09 (t, 1H), 8.35 (d, 1H), 8.55 (t, 1H), 8.86 (s, 1H), 9.08 (d, 1H).

O-(Diphenylmethyl)-N-(1-methyl-2-pyridinemethylene) hydroxylamine chloride O-(p-chlorobenzyl)-N-(1-methyl-2-pyridinemethylene) hydroxylamine chloride O-(p-methoxybenzyl)-N-(1-methyl-2-pyridinemethylene) hydroxylamine chloride O-(1-naphthylmethyl)-N-(1-methyl-2-pyridinemethylene) hydroxylamine chloride O-(2-methyl-1-naphthylmethyl)-N-(1-methyl-2-pyridinemethylene) hydroxylamine chloride, O-(2,3,4-trimethoxybenzyl)-N-(1-methyl-2-pyridinemethylene) hydroxylamine chloride, and O-(3,3-diphenylpropyl)-N-(1-methyl-2-pyridinemethylene) hydroxylamine chloride can be made by the same procedure replacing O-benzyl hydroxylamine in the above example respectively with O-(diphenylmethyl) hydroxylamine, O-(p-chlorobenzyl) hydroxylamine, O-(p-methoxybenzyl) hydroxylamine, O-(1-naphthylmethyl) hydroxylamine, O-(2-methyl-1-naphthylmethyl) hydroxylamine, O-(2,3,4-trimethoxybenzyl) hydroxylamine, and O-(3,3-diphenylpropyl) hydroxylamine.

EXAMPLE 2

Preparation of O-Benzyl-N-(3-hydroxy-1-methyl-2-pyridinemethylene)hydroxylamine chloride (1b).

O-Benzyl-N-(3-hydroxy-2-pyridinemethylene) hydroxylamine. A solution of O-benzyl hydroxylamine was prepared by dissolving 2 g of sodium hydroxide (0.05 mol) and 8 g of O-benzyl hydroxylamine hydrochloride (0.05 mol) in 50 mL of 50% ethanol-water. To this solution was added 6.15 g of 2-formyl-3-hydroxypyridine (0.05 mol). The mixture was stirred for 3 h at room temperature. The precipitate was then filtered and air dried to yield 10.3 g (90.3%) of pure product; m.p. 64°-65° C. ¹H NMR (CDCl₃) δ: 9.72 (s, 1H, OH); 8.37 (s, 1H, CH=N); 8.13 (d of d, J=2H, J=4Hz, 1H); 7.33 (m, 7H); 5.17 (s, 2H, CH₂). Anal. Calcd. for C₁₃H₁₂N₂O₂: C, 68.41; H, 5.30; N, 12.27. Found: C, 68.34; H, 5.32; N, 12.21.

O-Benzyl-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride. O-Benzyl-N-(3-hydroxy-2-

pyridinemethylene) hydroxylamine (2.65 g, 11.6 mmol) was placed in a pressure tube together with 7 g of methyl iodide and 40 mL of a 2:3 mixture of EtOH/Water. The reaction mixture was heated at 65° C. for 20 h and the solvent was then removed in vacuo. The residue was triturated with acetone to afford 2.50 g (58%) of product as greenish crystals; m.p. 247° C. (dec.). The methiodide salt (4.90 g, 13.2 mmol) was dissolved in 22 mL of a 1:1 mixture of acetonitrile and water and freshly made AgCl was then added (from 4.0 g AgNO₃ and excess conc. HCl, washed well with water). Additional water (15 mL) was added to this suspension and the resulting mixture was stirred at room temperature for 40 min. The silver iodide was removed by filtration and the filtrate was concentrated to dryness in vacuo to yield a residue that was triturated with acetone. The solid that resulted was washed well with acetone and dried to yield 3.61 g (98%) of product as white crystals; m.p. 146° C. ¹H NMR (DMSO-d₆) δ: 8.58 (d, 1H, J=5.0 Hz), 8.55 (s, 1H), 8.20 (d, 1H, J=7.3 Hz), 7.90 (d of d, 1H), 7.42 (m, 5H), 5.34 (s, 2H), 4.27 (s, 3H). Anal. Calcd. for C₁₄H₁₃N₂O_{1.5}Cl_{1.5}: C, 56.65; H, 5.78; N, 9.43; Cl, 11.94. Found: C, 56.65; H, 5.79; N, 9.40; Cl, 11.88.

O-(1-Naphtylmethyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride, O-(2-methyl-1-naphtylmethyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride, O-(2,3,4-trimethoxybenzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride, and O-(3,3-diphenylpropyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride can be made by the same procedure replacing O-benzyl hydroxylamine in the above example respectively with O-(1-naphtylmethyl) hydroxylamine, O-(2-methyl-1-naphtylmethyl) hydroxylamine, O-(2,3,4-trimethoxybenzyl) hydroxylamine, and O-(3,3-diphenylpropyl) hydroxylamine. O-(1-Naphtylmethyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride, O-(2-methyl-1-naphtylmethyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride, O-(2,3,4-trimethoxybenzyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride, and O-(3,3-diphenylpropyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride can be made by the same procedure substituting 3-hydroxy-2-pyridine carbaldehyde for 2-formyl-5-hydroxy pyridine.

EXAMPLE 3

Preparation of O-Methyl-N-(3-hydroxy-1-methyl-2-pyridinemethylene)hydroxylamine chloride (1c).

O-Methyl-N-(3-hydroxy-2-pyridinemethylene) hydroxylamine. NaOH (1.62 g, 0.04 mol) was added to a solution of methoxyamine hydrochloride (3.4 g, 0.04 mol) in 30 mL of H₂O. 3-Hydroxy-2-pyridine carboxaldehyde (5.0 g, 0.04 mol) was added to the clear solution, followed by 15 mL of EtOH. The reaction mixture was stirred at room temperature for 15 h and concentrated in vacuo to a minimum volume (approx. 5 mL). Water (15 mL) was added to this residue and the resulting solution was extracted with EtOAc (4×20 mL). The organic extract was dried (MgSO₄), filtered and concentrated in high vacuum to a yellow oil that crystallizes upon cooling in a bath of isopropanol-dry ice to yield 4.83 g (79%) of product as a white solid; m.p. 186°-7° C. (dec.). ¹H NMR (CDCl₃) δ: 9.82 (s, 1H); 8.35 (s, 1H); 8.21 (d of d, 1H, J=1.5 Hz, J=4.5 Hz); 7.30 (d of d, 1H, J=1.5 Hz, J=8.5 Hz); 7.19 (d of d, 1H, J=4.5 Hz, J=8.5 Hz).

O-Methyl-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine iodide. Methyl iodide (8.9 mL, 0.14 mol)

was added to a solution of O-methyl-N-(3-hydroxy-2-pyridinemethylene) hydroxylamine (4.83 g, 32 mmol) in 20 mL of acetonitrile. The reaction mixture was heated in a pressure glass bottle at 70° C. for 20 h and then concentrated to dryness in vacuo. The resulting yellow solid was triturated with acetone and filtered to yield 7.72 g (82%) of product which was used in the next step without further purification.

O-Methyl-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride. The methiodide obtained in the previous step (7.72 g, 26 mmol) was dissolved in 230 mL of H₂O. Freshly made AgCl (7.6 g, 53 mmol) was added to this solution and the resulting suspension was stirred at room temperature for 15 h. The reaction mixture was filtered and the filtrate was concentrated to dryness in vacuo. The white solid that resulted was triturated with warm (40° C.) acetone and filtered to yield 4.35 g (83%) of product; m.p. 186°-7° C. (dec.). ¹H NMR (DMSO-d₆) δ: 8.62 (d, 1H, J=6.0 Hz); 8.48 (s, 1H); 8.31 (d, 1H, J=8.5 Hz); 7.93 (d of d, 1H, J=6.0 Hz, J=8.5 Hz). Anal. Calcd. for C₈H₁₁N₂ClO₂: C, 47.41; H, 5.47; N, 13.83; Cl, 17.50. Found: C, 47.47; H, 5.49; N, 13.76; Cl, 17.43.

O-Methyl-N-(5-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride can be made by the same procedure replacing 3-hydroxy-2-pyridine aldehyde in the above example with 2-formyl-5-hydroxy pyridine. O-[2-(1-Methyl-1-piperidinium)ethyl]-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine dichloride and O-[2-(trimethyl ammonium)ethyl]-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine dichloride can be made by the same procedure replacing methoxyamine in the above example with: O-[2-(1-piperidine)ethyl] hydroxylamine and O-[2-(dimethylamino)ethyl] hydroxylamine.

EXAMPLE 4

Preparation O-(p-Chlorobenzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride (1d).

O-(p-Chlorobenzyl)hydroxylamine hydrochloride. The hydroxylamine hydrochloride was prepared by hydrazinolysis of the corresponding benzyloxyphtalimide. The phthalimide (15 g, 0.051 mol) was dissolved in DMF (75 mL) and MeOH (260 mL) and warmed to 60° C. The solution was treated with hydrazine monohydrate (5.7 g, 0.11 mol) and allowed to cool to room temperature for 3 h. The mixture was acidified to pH 2 with 2N HCl and filtered. The filtrate was evaporated to dryness and treated with 2N NaOH (75 mL). The oily product was extracted with ether, and the combined ether extracts were washed with H₂O, dried over anhydrous K₂CO₃, and concentrated in vacuo. The resulting oil was treated with ethereal hydrogen chloride to precipitate the product as a white solid. Recrystallization from EtOH gave the pure hydroxylamine hydrochloride as shiny white plates (9.5 g, 96%), m.p. 235° C. [lit. m.p. 245° C.; Schumann, E. L., Heinzelman, R. V., Greig, M. E., and Veldkamp, W., *J. Med. Chem.* 1964, 7, 329]. ¹H NMR (CDCl₃) δ: 7.23(s, 4H); 5.00(s, 2H).

O-(p-Cyanobenzyl)hydroxylamine hydrochloride, O-(2-Hydroxy-4-nitrobenzyl) hydroxylamine hydrochloride, and O-(p-Carboxy benzyl)hydroxylamine hydrochloride can be made by the same procedure replacing p-chlorobenzoyloxyphtalimide respectively with p-cyanobenzoyloxyphtalimide, 2-hydroxy-4-nitrobenzoyloxyphtalimide, and p-carboxybenzyl oxyphthalimide.

O-(p-Chlorobenzyl)-N-(3-hydroxy-2-pyridinemethylene) hydroxylamine. O-(4-Chlorobenzyl) hydroxylamine hydrochloride (9.75 g, 50 mmol) was neutralized with NaOH (2.0

g, 50 mmol dissolved in 20 mL H₂O). Ethanol (20 mL) was added to solubilize the resulting hydroxylamine. 3-Hydroxy-2-pyridinealdehyde (6.2 g, 50 mmol) dissolved in H₂O (150 mL) was added and the mixture heated on a steam bath for 30 min. The solution was cooled to room temperature. The product crystallized out as long pale yellow needles. The mixture was filtered to isolate the pure product (12.5 g, 96%), m.p. 89°–90° C. ¹H NMR(CDCl₃) d: 9.67 (s, 1H); 8.33 (s, 1H); 7.33–7.13 (m, 7H); 5.17 (s, 2H). Anal. Calcd. for C₁₃H₁₁N₂O₂Cl: C, 59.43; H, 4.23; N, 10.67; Cl, 13.49. Found: C, 59.43; H, 4.26; N, 10.64; Cl, 13.56.

O-(p-Chlorobenzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine iodide. A solution of O-(p-chlorobenzyl)-N-(3-hydroxy-2-pyridinemethylene) hydroxylamine (1.0 g, 3.8 mmol) in EtOH (45 mL) was placed in a glass pressure tube. Methyl iodide (1 mL, 16.0 mmol) was then added, and the tube was sealed tightly. The tube was heated at 69° C. in an oil bath for 20 h. The solution was concentrated in vacuo. The oily residue was triturated with acetone to give the crude product as a yellow solid (1.46 g, 95%). Recrystallization from acetone gave the pure product as a shiny yellow solid (0.8 g, 52%), m.p. 164°–166° C. ¹H NMR (CDCl₃/DMSO-d₆) d: 8.83–8.67 (m, 2H); 8.13–7.67 (m, 2H); 7.33 (s, 4H); 5.00 (s, 2H); 4.43 (s, 3H). Anal. Calcd. for C₁₄H₁₄N₂O₂ClI: C, 41.55; H, 3.49; N, 6.92; Cl, 8.76; I, 31.36. Found: C, 41.62; H, 3.53; N, 6.92; Cl, 8.71; I, 31.28.

O-(p-Chlorobenzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride. To a solution of the corresponding pyridinium iodide (1.0 g, 2.47 mmol) in CH₃CN/H₂O (1:1, 15 mL) was added freshly made AgCl (from 0.54 g AgNO₃ and conc. HCl). The mixture was allowed to stir at room temperature for 1.5 h. It was then filtered through a bed of Celite. The solvent was removed in vacuo. Trituration of the residue with acetone gave the pure pyridinium chloride (0.72 g) in 93% yield, m.p. 175° C. (dec.). ¹H NMR (CDCl₃/DMSO-d₆) d: 8.67 (m, 1H); 8.37 (m, 1H); 8.00–7.67 (m, 1H); 7.37 (s, 4H); 5.3 (s, 2H); 4.33 (s, 3H). Anal. Calcd. for C₁₄H₁₄N₂OCl₂: C, 53.68; H, 4.51; N, 8.95; Cl, 22.64. Found: C, 53.76; H, 4.56; N, 8.92; Cl, 22.57.

O-(p-Cyanobenzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride, O-(2-hydroxy-4-nitrobenzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride, and O-(p-carboxybenzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride can be made by the same procedure replacing O-(p-chlorobenzyl) hydroxylamine respectively with O-(p-cyanobenzyl)-N-(3-hydroxy-2-pyridinemethylene) hydroxylamine, O-(2-hydroxy-4-nitrobenzyl)-N-(3-hydroxy-2-pyridinemethylene) hydroxylamine, and O-(p-carboxybenzyl)-N-(3-hydroxy-2-pyridinemethylene) hydroxylamine. O-(p-Chlorobenzyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride, O-(p-cyanobenzyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride, O-(2-hydroxy-4-nitrobenzyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride, and O-(p-carboxybenzyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride can be made by the same procedure replacing 3-hydroxy-2-pyridine aldehyde with 2-formyl-5-hydroxy pyridine.

EXAMPLE 5

Preparation of O-(p-Methoxybenzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride (1e).

O-(p-Methoxybenzyl)-N-(3-hydroxy-2-pyridinemethylene) hydroxylamine. O-(p-methoxybenzyl) hydroxylamine (5.55 g, 29 mmol; Grochowski et al, 1976

and Schumann et al, 1964, vide supra) was added to a solution of NaOH (1.2 g, 29 mmol) in 15 mL H₂O. 3-Hydroxy-2-pyridine carbaldehyde (3.6 g, 29 mmol) was then added, followed by 15 mL of 95% EtOH and 10 mL of acetone. The reaction mixture was stirred at room temperature for 4 h and was then poured over 400 mL of crashed ice. A beige solid comes out of solution upon scratching the sides of the beaker. It was filtered, washed with water and dried to yield 7.03 g (93%) of product; m.p. 79°–81° C. ¹H NMR (CDCl₃) d: 9.85 (s, 1H); 8.38 (s, 1H); 8.19 (d of d, 1H); 7.36 (d of d, 2H); 7.28 (d of d, 1H); 7.18 (d of d, 1H); 6.93 (d of d, 2H); 5.15 (s, 2H); 3.82 (s, 3H).

O-(p-Methoxybenzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride. Methyl iodide (7.2 mL, 0.12 mol) was added to a solution of O-(p-methoxybenzyl)-N-(3-hydroxy-2-pyridinemethylene) hydroxylamine (3.72 g, 14 mmol) in 60 mL of acetonitrile. The resulting mixture was divided into three pressure glass vessels and heated at 70° C. for 3 days. The solvent was removed in vacuo and the residue was triturated with ether/acetone (3:1); after drying, the methiodide weighed 4.88 g (85%). It was dissolved in 500 mL H₂O and 300 mL of acetonitrile and freshly made AgCl (2 eq.) was then added. The suspension was stirred at room temperature for 1.5 h and then filtered through Celite. The filtrate was concentrated to dryness in vacuo and the residue was triturated with acetone/ether (1:1). The yellow solid was filtered, washed first with acetone/ether (1:1), then with ether and dried to yield 3.57 g (97%) of pure product, m.p. 183°–184° C. (dec.). ¹H NMR (d₆-DMSO) d: 8.57 (d, 1H, J=6.1 Hz); 8.50 (s, 1H); 8.19 (d, 1H, J=8.2 Hz); 7.88 (d of d, 1H, J=6.1 Hz, J=8.2 Hz); 7.38 (d, 2H, J=8.7 Hz); 6.95 (d, 2H, J=8.7 Hz); 5.25 (s, 2H); 4.26 (s, 3H); 3.75 (s, 3H). Anal. Calcd. for C₁₅H₁₇ClN₂O₃ x 0.55 H₂O: C, 6.53; H, 5.73; N, 8.79; Cl, 11.13. Found: C, 56.53; H, 5.72; N, 8.79; Cl, 11.18.

O-(p-Dimethylaminocarbonyloxybenzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride, O-(p-propylaminocarbonylbenzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride, and O-(p-ethoxycarbonylbenzyl)-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride can be made by the same procedure replacing O-(p-methoxybenzyl) hydroxylamine in the above example respectively with O-(p-dimethylaminocarbonyloxybenzyl) hydroxylamine, O-(p-propylaminocarbonylbenzyl) hydroxylamine, and O-(p-ethoxycarbonylbenzyl) hydroxylamine. O-(p-Methoxybenzyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride, O-(p-dimethylaminocarbonyloxybenzyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride, O-(p-propylaminocarbonylbenzyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride, and O-(p-ethoxycarbonylbenzyl)-N-(5-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine chloride can be made by the same procedure replacing 3-hydroxy-2-pyridine aldehyde in the above example with 2-formyl-5-hydroxy pyridine.

EXAMPLE 6

Preparation of O-Benzyl-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine chloride (2a).

O-Benzyl-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-2-pyridinemethylene] hydroxylamine. Dimethylcarbamyl chloride (7 mL, 76 mmol) was added to a solution of 2.59 g (11 mmol) of O-benzyl-N-(3-hydroxy-2-pyridinemethylene) hydroxylamine (prepared as reported on previous page) in 20 mL of pyridine. The resulting mixture was stirred at 65° C. for 12 h and poured over 400 mL of ice water. The aqueous solution was extracted with ether (6x50

mL); the ether layer was dried (Na_2SO_4), filtered and concentrated to dryness to a yellow oil which was coevaporated with H_2O and with acetone to remove the remaining pyridine. The residue was dissolved in 10 mL acetone, and water was added with cooling and stirring until no more precipitate came out of solution. The white solid was filtered, washed with water and dried to yield 3.02 g (89%) of product; m.p. $65^\circ\text{--}66^\circ\text{C}$. ^1H NMR (CDCl_3) δ : 8.54 (d of d, 1H, $J=4\text{Hz}$, $J=1\text{Hz}$); 8.38 (s, 1H); 7.54 (d of d, 1H, $J=8.3\text{Hz}$, $J=1.5\text{Hz}$); 7.30–7.43 (m, 6H); 5.30 (s, 2H); 3.06 (s, 3H); 2.97 (s, 3H).

O-Benzyl-N-[3-(N,N' -dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine iodide. A solution of 2.95 g (9.9 mmol) of O-benzyl-N-[3-(N,N' -dimethylcarbamoyl)hydroxy-2-pyridinemethylene] hydroxylamine and 2.8 mL (44 mmol) of methyl iodide in 20 mL of acetonitrile was heated at 65°C in a glass pressure bottle for 2 days. The reaction mixture was concentrated to dryness to a dark orange oil which was triturated with cold acetone to yield a bright yellow precipitate that was washed with acetone and ether. After drying the product weighed 4.02 g (92%).

O-Benzyl-N-[3-(N,N' -dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine chloride. The methiodide obtained in the previous step (4.02 g, 9 mmol) was dissolved in 25 mL H_2O and 20 mL acetonitrile. Freshly prepared AgCl (2.61 g, 18 mmol) was added to this solution and the resulting suspension was stirred at room temperature for 1 h. The reaction mixture was filtered and the filtrate was concentrated to dryness to a white solid that was triturated with warm (40°C) acetone for 20 min and filtered. The white solid was washed with acetone and ether to yield 3.2 g (quantitative) of the pure chloride, m.p. $150^\circ\text{--}152^\circ\text{C}$ (dec.); ^1H NMR ($\text{DMSO}-d_6$) δ : 9.09 (d, $J=5.5\text{Hz}$, 1H); 8.74 (s, 1H); 8.59 (d, $J=8.4\text{Hz}$, 1H); 8.19 (d of d, $J=8.4\text{Hz}$, $J=5.5\text{Hz}$, 1H); 7.42 (m, 5H); 5.33 (s, 2H); 4.39 (s, 3H); 2.98 (s, 3H); 2.92 (s, 3H). Anal. Calcd. for $\text{C}_{17}\text{H}_{20}\text{ClN}_3\text{O}_3 \times 0.45 \text{H}_2\text{O}$: C, 57.04; H, 5.89; N, 11.74; Cl, 9.91. Found: C, 56.99; H, 5.95; N, 11.69; Cl, 9.91.

O-Benzyl-N-[3-(N,N' -diethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine chloride and O-benzyl-N-[3-(N,N' -difluoroethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine chloride can be made by the same procedure replacing N,N -dimethylcarbamoyl chloride respectively with N,N -diethylcarbamoyl chloride and N,N -difluoroethylcarbamoyl chloride. O-Benzyl-N-[5-(N,N' -dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine chloride. O-benzyl-N-[5-(N,N' -diethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine chloride and O-benzyl-N-[5-(N,N' -difluoroethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine chloride can be made by the same procedure replacing 3-hydroxy-2-pyridine aldehyde with 2-formyl-5-hydroxy pyridine. O-[2-(1-Methyl-1-piperidinium)ethyl]-N-[3-(N,N' -dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine dichloride, and O-[2-(trimethylammonium)ethyl]-N-[3-(N,N' -dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine dichloride can be made by the same procedure replacing O-benzylhydroxylamine in the above example with O-[2-(1-piperidine)ethyl] hydroxylamine and O-[2-(dimethylamino)ethyl] hydroxylamine.

EXAMPLE 7

Preparation of O-(Diphenylmethyl)-N-[3-(N,N' -dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine chloride (2b).

O-(Diphenylmethyl)-N-(3-hydroxy-2-pyridinemethylene] hydroxylamine. O-Diphenylmethyl

hydroxylamine (3.4 g, 14.4 mmol; Grochowski et al, 1976 and Schumann et al, 1964, vide supra) was added to a solution of NaOH (0.58 g, 14.4 mmol) in 7 mL of water. 3-Hydroxy-2-pyridine carbaldehyde (1.8 g, 14.4 mmol) was added to this solution and the resulting mixture was stirred at room temperature for 4 h. The white solid in the reaction mixture was filtered, washed with $\text{H}_2\text{O}/\text{EtOH}$ (1:1) and dried to yield 3.17 (72%) of product, m.p. $82^\circ\text{--}83^\circ\text{C}$. ^1H NMR (CDCl_3) δ : 9.60 (s, 1H); 8.51 (s, 1H); 8.2 (d of d, 1H); 7.30–7.43 (m, 10H); 7.23 (d of d, 1H); 7.16 (d of d, 1H); 6.29 (s, 1H).

O-(Diphenylmethyl)-N-[3-(N,N' -dimethylcarbamoyl)hydroxy-2-pyridinemethylene] hydroxylamine. Dimethylcarbamyl chloride (9 mL, 98 mmol) was added to a solution of O-(diphenylmethyl)-N-(3-hydroxy-2-pyridinemethylene] hydroxylamine (3.0 g, 9.9 mmol) in 30 mL of pyridine. The resulting mixture was stirred at room temperature for 14 h and poured over 400 mL of crushed ice. A white solid precipitates upon scratching the sides of the beaker. It was filtered, washed thoroughly with water and dried to yield 3.47 g (94%) of product, m.p. $89^\circ\text{--}90^\circ\text{C}$. ^1H NMR (d_6 -DMSO) δ : 8.49 (d of d, 1H); 8.37 (s, 1H); 7.64 (d of d, 1H); 7.47 (d of d, 1H); 7.42–7.28 (m, 10H); 6.29 (s, 1H); 2.81 (s, 3H); 2.74 (s, 3H).

O-(Diphenylmethyl)-N-[3-(N,N' -dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine chloride. Methyl iodide (2.8 mL, 46 mmol) was added to a solution of O-(diphenylmethyl)-N-[3-(N,N' -dimethylcarbamoyl)hydroxy-2-pyridinemethylene] hydroxylamine (3.44 g, 9.2 mmol) in 40 mL of acetonitrile. The resulting mixture was divided into two pressure glass vessels and heated at 70°C for 3 days. The solvent was removed in vacuo and the residue (4.92 g) was dissolved in a mixture of acetonitrile/water (200 mL:200 mL). Freshly made AgCl (2 eq, washed well with H_2O) was added to this solution and the resulting suspension was stirred at room temperature for 2 h. It was filtered through Celite and the filtrate was concentrated to dryness in vacuo to a residue that was triturated with ether. The white solid was collected by filtration and dried to yield 3.84 g (98%) of pure product, m.p. $153^\circ\text{--}154^\circ\text{C}$ (dec.). ^1H NMR (d_6 -DMSO) δ : 9.06 (d, 1H, $J=6.0\text{Hz}$); 8.87 (s, 1H); 8.55 (d, 1H, $J=8.4\text{Hz}$); 8.18 (d of d, 1H, $J=6.0\text{Hz}$, $J=8.4\text{Hz}$); 7.45–7.30 (m, 10H); 6.44 (s, 1H); 4.35 (s, 3H); 2.8 (s, 3H); 2.79 (s, 3H). Anal. Calcd. for $\text{C}_{23}\text{H}_{24}\text{ClN}_3\text{O}_3 \times 1.19 \text{H}_2\text{O}$: C, 61.76; H, 5.94; N, 9.39; Cl, 7.93. Found: C, 61.76; H, 6.02; N, 9.41; Cl, 7.97.

O-(Diphenylmethyl)-N-[3-(N' -isopropylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine chloride and O-(diphenylmethyl)-N-[3-(N' -2-fluoroethyl)carbamoyl]hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine chloride can be made by the same procedure replacing N,N -dimethylcarbamyl chloride respectively with isopropyl isocyanate and 2-fluoroethyl isocyanate. O-(Diphenylmethyl)-N-[5-(N' -isopropylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine chloride and O-(diphenylmethyl)-N-[5-(N' -2-fluoroethyl)carbamoyl]hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine chloride can be made by the same procedure replacing 3-hydroxy-2-pyridine aldehyde with 2-formyl-5-hydroxy pyridine.

EXAMPLE 8

Preparation of O-(p-Methoxybenzyl)-N-[3-(N,N' -dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine chloride (2c).

O-(p-Methoxybenzyl)-N-[3-(N,N' -dimethylcarbamoyl)hydroxy-2-pyridine methylene] hydroxylamine. Dimethyl-

carbamyl chloride (10 mL, 0.11 mol) was added to a solution of O-(p-methoxybenzyl)-N-(3-hydroxy-2-pyridinemethylene) hydroxylamine (3.3 g, 12.8 mmol) in 30 mL of pyridine. The resulting mixture was stirred at room temperature for 14 h and then poured over 400 mL of crushed ice. Since it was not possible to induce precipitation of the product, the aqueous mixture was extracted with ether (4 x 100 mL) and the organic extract was concentrated to dryness in vacuo. The oily residue was coevaporated with water to remove traces of pyridine and then with acetone. The 300 MHz ¹H NMR of the resulting yellow oil shows no pyridine or water and was used in the next step without further purification.

O-(p-Methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine chloride. Methyl iodide (2.9 mL, 46.4 mmol) was added to each of two pressure glass tubes containing a solution of O-(p-methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-2-pyridinemethylene] hydroxylamine (3.82 g, 11.6 mmol, divided into two) in 40 mL of acetonitrile. The sealed reaction vessels were heated at 70° C. for 3 days and then the solvent was removed in vacuo. The resulting oil crystallizes upon standing at 0°-5° C. for 14 h in a 2:1 mixture of acetone/ether (75 mL). The yellow solid was filtered, washed with ether and dried to yield 4.94 g (90%) of crude methiodide. It was dissolved in 1:1 mixture of H₂O/ acetonitrile (200 mL) and then freshly made AgCl (21 mmol, washed well with water) was added. The resulting suspension was stirred at room temperature for 2 h and filtered through Celite. The filtrate was concentrated to dryness in vacuo and the residue was triturated with 1:1 acetone/ether. The white solid that resulted was filtered, washed with ether and dried to yield 3.72 g (84.4%) of pure chloride, m.p. 145°-6° C. (dec.). Anal. Calcd. for C₁₈H₂₂ClN₃O₄ x 0.5 H₂O: C, 55.59; H, 5.96; N, 10.81; Cl, 9.12. Found: C, 55.59; H, 6.03; N, 10.76; Cl, 9.17.

O-(p-Methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-ethyl-2-pyridinemethylene] hydroxylamine chloride. O-(p-methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-benzyl-2-pyridinemethylene] hydroxylamine bromide, O-(p-methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-(1-naphthylmethyl)-2-pyridinemethylene] hydroxylamine chloride, O-(p-methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-(2-methyl-1-naphthylmethyl)-2-pyridinemethylene] hydroxylamine chloride, O-(p-methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-diphenylmethyl-2-pyridinemethylene] hydroxylamine chloride, O-(p-methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-(2-hydroxy-4-nitrobenzyl)-2-pyridinemethylene] hydroxylamine chloride, O-(p-methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-(p-carboxybenzyl)-2-pyridinemethylene] hydroxylamine chloride, and O-(p-methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-(p-cyanobenzyl)-2-pyridinemethylene] hydroxylamine chloride can be made by the same procedure replacing methyl iodide in the above example respectively with ethyl iodide, benzyl bromide, 1-chloromethyl naphthalene, 1-chloromethyl-2-methyl naphthalene, chlorodiphenylmethane, 2-chloromethyl-4-nitrophenol, 4-chloromethyl benzoic acid, and 4-cyano benzyl chloride. O-(p-Methoxybenzyl)-N-[5-(N',N'-dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine chloride, O-(p-methoxybenzyl)-N-[5-(N',N'-dimethylcarbamoyl)hydroxy-1-ethyl-2-pyridinemethylene] hydroxylamine chloride, O-(p-methoxybenzyl)-N-[5-(N',N'-dimethylcarbamoyl)

hydroxy-1-benzyl-2-pyridinemethylene] hydroxylamine bromide, O-(p-methoxybenzyl)-N-[5-(N',N'-dimethylcarbamoyl)hydroxy-1-(1-naphthylmethyl)-2-pyridinemethylene] hydroxylamine chloride, O-(p-methoxybenzyl)-N-[5-(N',N'-dimethylcarbamoyl)hydroxy-1-(2-methyl-1-naphthylmethyl)-2-pyridinemethylene] hydroxylamine chloride, O-(p-methoxybenzyl)-N-[5-(N',N'-dimethylcarbamoyl)hydroxy-1-diphenylmethyl-2-pyridinemethylene] hydroxylamine chloride, O-(p-methoxybenzyl)-N-[5-(N',N'-dimethylcarbamoyl)hydroxy-1-(2-hydroxy-4-nitrobenzyl)-2-pyridinemethylene] hydroxylamine chloride, O-(p-methoxybenzyl)-N-[5-(N',N'-dimethylcarbamoyl)hydroxy-1-(p-carboxybenzyl)-2-pyridinemethylene] hydroxylamine chloride, O-(p-methoxybenzyl)-N-[5-(N',N'-dimethylcarbamoyl)hydroxy-1-(p-cyanobenzyl)-2-pyridinemethylene] hydroxylamine chloride can be made by the same procedure replacing 3-hydroxy-2-pyridine aldehyde with 2-formyl-5-hydroxy pyridine.

EXAMPLE 9

Preparation of N-[5-(N',N'-Dimethylcarbamoyl)hydroxy-1-methyl-2-pyridine methylene] hydroxylamine chloride (2d).

2-Formyl-5-hydroxypyridine. To a stirred solution of 21.8 g of 5-hydroxy-2-picoline (0.2 mol) in 200 mL of glacial acetic acid was added 18 mL of 30% hydrogen peroxide (0.159 mol) in one portion. The mixture was heated in an oil bath at 80°-85° C. with stirring for 3 h. Then another 18 mL of hydrogen peroxide was added and the mixture was stirred for 3 h at the same temperature. Excess solvent was removed in vacuo followed by the addition of acetone, which caused the pyridine N-oxide to crystallize. Without further purification, 200 mL of acetic anhydride was added to the solid and the mixture was heated at 120° C. with stirring in an oil bath for 2 h. After cooling to room temperature, excess acetic anhydride was removed by high vacuum distillation. The oily material was again oxidized with 30% hydrogen peroxide twice and rearranged with acetic anhydride following the same procedure and amounts depicted above. The black oily material obtained was hydrolyzed with 200 mL of 1N HCl (0.2 mol) at room temperature for 3 weeks (hydrolysis at higher temperature may be harmful to the pyridine nucleus). The mixture was neutralized with anhydrous sodium carbonate, indicated by litmus paper and then extracted three times with 300 mL of diethylether. The combined ether extracts were dried (MgSO₄), filtered and evaporated to leave a solid material in a small amount of oil. The pure product (7.8 g, 32%) was obtained by filtering the solid, followed by washing it with a small amount of ether. It is yellow in color and shows a sharp melting point at 186°-187° C. ¹H NMR (d₆-DMSO) δ: 11.10 (br. s, 1H, OH); 9.87 (s, 1H, CHO); 8.35 (d, J=2Hz, 1H); 7.58 (d, J=9 Hz, 1H); 7.35 (d of d, J=2Hz, 9Hz, 1H).

N-(5-Hydroxy-2-pyridinemethylene) hydroxylamine. To 200 mL of 2.5% (w/w) sodium hydroxide solution was dissolved 5.5 g of 5-hydroxy-2-formylpyridine (45 mmol). Subsequently 12.5 g of hydroxylamine HCl (180 mmol) was added to the solution in one portion. The solution turned cloudy after being stirred for 10 minutes. After stirring at room temperature for 3 h, the precipitate was filtered under vacuum and dried in the air for several days to yield 4.8 g of N-(5-hydroxy-2-pyridinemethylene) hydroxylamine (78%). The solid is off white and decomposes at 195° C. ¹H NMR (d₆-DMSO) δ: 11.27 (br. s, 1H); 10.83 (br. s, 1H); 8.18 (d, J=2 Hz, 1H); 8.05 (s, 1H, CH=N); 7.72 (d, J=9Hz, 1H); 7.25 (d of d, J=2Hz, 9Hz, 1H).

N-[5-(N',N'-Dimethylcarbamoyl)hydroxy-2-pyridine methylene] hydroxylamine. Dimethylcarbamoyl chloride

(5.2 mL, 57 mmol) was added to a solution of N-(5-hydroxy-2-pyridinemethylene) hydroxylamine (6.53 g, 47 mmol) in 40 mL of pyridine. It was stirred at room temperature overnight and then the solvent was removed in vacuo, leaving a light green paste. The paste was dissolved in a minimum quantity of acetone and then triturated with ethyl acetate and placed in the freezer overnight. Glassy needles formed and were filtered and washed with ethyl acetate to yield 3.0 g of pure product (30%), m.p. 134°–135° C.

N-[5-(N',N'-Dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine chloride. Methyl iodide (5.3 mL, 85 mmol) was added to a solution of N-[5-(N',N'-dimethylcarbamoyl)hydroxy-2-pyridine methylene] hydroxylamine (2.8 g, 13.4 mmol) in 25 mL of acetonitrile. The resulting mixture was put into a glass pressure vessel and heated at 80° C. for 3 days. The solvent was removed in vacuo and the residue was dissolved in a minimum quantity of acetone, triturated with ether and placed in the freezer overnight. A dark yellow solid resulted and was filtered under vacuum and washed with 1:1 ether/acetone to yield 3.25 g of the iodide salt. Freshly made AgCl (2 eq. washed well with water) was added to the iodide salt in acetonitrile/water (60 mL/100 mL) and stirred for several hours. After filtration, the residue was crystallized from acetone/ether and dried in the air for several days to yield 2.5 g of the chloride salt (monohydrate, 72% yield); m.p. 159°–160° C. ¹H NMR (d₆-DMSO) d: 9.18 (s, 1H); 8.65 (s, 1H); 8.40 (d of d, 2H, J=2 Hz, 9 Hz); 4.36 (s, 3H); 3.3 (s, H₂O); 3.1 (s, 3H); 2.95 (s, 3H). Anal. Calcd. for C₁₀H₁₄N₃O₃ClH₂O: C, 43.25; H, 5.81; N, 15.13; Cl, 12.76. Found: C, 43.42; H, 5.81; N, 15.09; Cl, 12.87.

N-[3-(N',N'-Dimethylcarbamoyl)hydroxy-1-methyl-2-pyridinemethylene] hydroxylamine chloride can be made by the same procedure replacing 2-formyl-5-hydroxypyridine with 3-hydroxy-2-pyridine carbaldehyde.

EXAMPLE 10

Preparation of 2-[[[Aminocarbonyl]hydrazono]methyl]-3-hydroxy-1-methyl pyridinium chloride (3a).

2-[[[Aminocarbonyl]hydrazono]methyl]-3-hydroxy pyridine. Sodium acetate (2.21 g, 16 mmol) was dissolved in 25 mL of water and then semicarbazide hydrochloride was added (1.81 g, 16 mmol), followed by 3-hydroxy-2-pyridine aldehyde (2.0 g, 16 mmol). The resulting mixture was stirred at room temperature for 2 h. The solid in the reaction mixture was then filtered, washed with water and dried to yield 2.71 g (93%) of the semicarbazone. It was used in the next step without further purification; m.p.: 215°–217° C. (dec.).

2-[[[Aminocarbonyl]hydrazono]methyl]-3-hydroxy-1-methyl pyridinium iodide. A glass pressure tube containing a stirring bar was charged with 20 grams (11.1 mmoles) of 2-[[[aminocarbonyl]hydrazono]methyl]-3-hydroxy pyridine, 2.5 g of barium carbonate, 7.0 g (49.3 mmol) of methyl iodide and 30 mL of DMF. The reaction mixture was stirred for two days at 65° C. in an oil bath. After that time the solvent was removed in vacuo, the residue shaken with 50 mL of warm water and filtered. The filtrate was then treated with an aqueous solution of H₂SO₄ in amount needed for the precipitation of Ba²⁺ present in the solution. After collecting BaSO₄, the filtrate was concentrated and crystallized from a mixture of water and ethanol to afford 2.1 g (58.3%) of the methiodide salt, m.p. 180°–182° C.

2-[[[Aminocarbonyl]hydrazono]methyl]-3-hydroxy-1-methyl pyridinium chloride. The preceding iodide, 6.0 grams (18.6 mmol) was dissolved in 150 mL of water and

stirred with silver chloride precipitated from a solution of 6.0 g (35.3 mmol) of silver nitrate with excess of aqueous HCl. After 40 min the reaction mixture was filtered and the precipitate washed with a total of 100 mL of warm water. Pale yellow crystals of product melting at 230°–232° C. (dec.) were obtained from the combined filtrate by both rotoevaporation and precipitation with acetone. Yield 4.12 grams (95.3%). ¹H NMR (D₂O, TSP) d: 8.45 (s, 1H), 8.36 (d, 1H, J=5.8 Hz), 8.03 (d, 1H, J=8.3 Hz), 7.80 (d of d, 1H), 4.42 (s, Me). Anal. Calcd for C₈H₁₁N₄O₂Cl: C, 41.66; H, 4.81; N, 24.28; Cl, 15.37. Found C, 41.59, H, 4.85; N, 24.28; Cl, 15.44.

2-[[[Aminothiocabonyl]hydrazono]methyl]-3-hydroxy-1-methyl pyridinium chloride. 2-hydrazonomethyl-3-hydroxy-1-methyl pyridinium chloride, 1-methyl-2-(3-hydroxy-1-methyl-2-pyridinemethylene) hydrazine chloride, 1-methyl-1-phenyl-2-(3-hydroxy-1-methyl-2-pyridinemethylene) hydrazine chloride, 1,1-diphenyl-2-(3-hydroxy-1-methyl-2-pyridinemethylene) hydrazine chloride, 1-(2,4-dinitrophenyl)-2-(3-hydroxy-1-methyl-2-pyridinemethylene) hydrazine chloride, and 1-(2-naphtyl)-2-(3-hydroxy-1-methyl-2-pyridinemethylene) hydrazine chloride can be made by the same procedure replacing semicarbazide in the above example respectively with thiosemicarbazide, hydrazine, methylhydrazine, 1-methyl-1-phenylhydrazine, 1,1-diphenylhydrazine, 2,4-dinitrophenylhydrazine, and 2-naphtylhydrazine. 2-[[[Aminocarbonyl]hydrazono]methyl]-5-hydroxy-1-methyl pyridinium chloride, 2-[[[aminothiocabonyl]hydrazono]methyl]-5-hydroxy-1-methyl pyridinium chloride, 2-hydrazonomethyl-5-hydroxy-1-methyl pyridinium chloride, 1-methyl-2-(5-hydroxy-1-methyl-2-pyridinemethylene) hydrazine chloride, 1-methyl-1-phenyl-2-(5-hydroxy-1-methyl-2-pyridinemethylene) hydrazine chloride, 1,1-diphenyl-2-(5-hydroxy-1-methyl-2-pyridinemethylene) hydrazine chloride, 1-(2,4-dinitrophenyl)-2-(5-hydroxy-1-methyl-2-pyridinemethylene) hydrazine chloride, and 1-naphtyl-2-(5-hydroxy-1-methyl-2-pyridinemethylene) hydrazine chloride can be made by the same procedure replacing 3-hydroxy-2-pyridine aldehyde with 2-formyl-5-hydroxy pyridine. 2-Hydrazonomethyl-3-hydroxy-1,6-dimethyl pyridinium chloride, 1-methyl-2-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydrazine chloride, 1-methyl-1-phenyl-2-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydrazine chloride, and 1,1-diphenyl-2-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydrazine chloride can be made by the same procedure replacing 3-hydroxy-2-pyridine aldehyde in the above example with 3-hydroxy-6-methyl-2-pyridine aldehyde.

EXAMPLE 11

Preparation of 3-Hydroxy-1-methyl-2-[[[N-phenylaminocarbonyl]hydrazono]methyl]pyridinium chloride (3b).

3-Hydroxy-2-[[[N-phenylaminocarbonyl]hydrazono]methyl] pyridine. 4-Phenyl semicarbazide (4.91 g, 33 mmol) was added to a suspension of 3-hydroxy-2-pyridine carbaldehyde (4.0 g, 33 mmol) in a mixture of ethanol water (30 mL:40 mL). The reaction mixture was stirred at room temperature for 3 h and the yellow product was collected by filtration. The yellow precipitate was washed with EtOH/H₂O 1:1 and dried under P₂O₅ to yield 8.22 g (99%) of product. It can be recrystallized from EtOH/H₂O; m.p. 201°–2° C. (dec.). ¹H NMR (d₆-DMSO) d: 10.85 (br.s., 1H); 10.6 (br.s., 1H); 9.14 (s, 1H); 8.27 (s, 1H); 8.15 (d of d, 1H); 7.55 (d, 2H); 7.26–7.36 (m, 4H); 7.01 (t, 1H).

3-Hydroxy-2-[[N-phenylaminocarbonyl]hydrazono]methyl-1-methyl pyridinium chloride. Methyl iodide (7.2 mL, 0.12 mol) was added to a suspension of 2-[[N-phenylaminocarbonyl]hydrazono]methyl-3-hydroxy pyridine (3.72 g, 15 mmol) in 50 mL of acetonitrile. The reaction mixture was placed in two pressure glass vessels and heated at 70° C. for 3 days. The solid which filled the reaction flasks was filtered and triturated well with warm (40° C.) acetone. The product was dried to yield 5.13 g (89%) of methiodide. It was dissolved in a mixture of water/acetonitrile (500 mL:300 mL) and then freshly made AgCl (2 eq) was added. The suspension was stirred at room temperature for 2 h and then filtered through Celite. The filtrate was concentrated to dryness in vacuo and the residue was triturated with acetone. The yellow solid was filtered, washed with acetone and dried to yield 3.67 g (93%) of the pure chloride; m.p. 220°–221° C. (dec.). It can be recrystallized from EtOH. ¹H NMR (d₆-DMSO) δ: 11.74 (br.s., 1H); 9.27 (s, 1H); 8.58 (d, 1H, J=5.7 Hz); 8.39 (s, 1H); 8.17 (d, 1H, J=8.2 Hz); 7.85 (d of d, 1H, J=5.7 Hz, J=8.2 Hz); 7.57 (d, 2H, J=7.6 Hz); 7.31 (t, 2H, J=7.6 Hz); 7.04 (t, 1H, J=7.6 Hz); 4.48 (s, 3H). Anal. Calcd. for C₁₄H₁₅ClN₄O₂ x 0.95 H₂O: C, 51.90; H, 5.26; N, 17.29; Cl, 10.94. Found: C, 51.90; H, 5.22; N, 17.36; Cl, 10.89.

3-Hydroxy-1-methyl-2-[[N-phenylaminothiocarbonyl]hydrazono]methylpyridinium chloride, 2-[[N,N-diphenylaminocarbonyl]hydrazono]methyl-3-hydroxy-1-methyl pyridinium chloride, 2-[[N,N-dimethylaminothiocarbonyl]hydrazono]methyl-3-hydroxy-1-methyl pyridinium chloride, 2-[[N-ethylaminothiocarbonyl]hydrazono]methyl-3-hydroxy-1-methyl pyridinium chloride, 3-hydroxy-1-methyl-2-[[N-naphthylaminocarbonyl]hydrazono]methyl pyridinium chloride, and 2-[[N-(1-chloro-2-naphthyl)amino carbonyl]hydrazono]methyl-3-hydroxy-1-methyl pyridinium chloride can be made by the same procedure replacing 4-phenylsemicarbazide in the above example respectively with 4-phenyl-3-thiosemicarbazide, 4,4-diphenylsemicarbazide, 4,4-dimethyl-3-thiosemicarbazide, 4-ethyl-3-thiosemicarbazide, 4-(2-naphthyl)semicarbazide and 4-(1-chloro-2-naphthyl)semicarbazide. 5-Hydroxy-1-methyl-2-[[N-phenylaminocarbonyl]hydrazono]methyl pyridinium chloride, 5-hydroxy-1-methyl-2-[[N-phenylaminothiocarbonyl]hydrazono]methyl pyridinium chloride, 2-[[N,N-di phenylaminocarbonyl]hydrazono]methyl-5-hydroxy-1-methyl pyridinium chloride, 2-[[N,N-dimethylaminothiocarbonyl]hydrazono]methyl-5-hydroxy-1-methyl pyridinium chloride, 2-[[N-ethylaminothiocarbonyl]hydrazono]methyl-5-hydroxy-1-methyl pyridinium chloride, 5-hydroxy-1-methyl-2-[[N-naphthylaminocarbonyl]hydrazono]methyl pyridinium chloride, and 2-[[N-(1-chloro-2-naphthyl)amino carbonyl]hydrazono]methyl-5-hydroxy-1-methyl pyridinium chloride can be made by the same procedure replacing 3-hydroxy-2-pyridine aldehyde with 2-formyl-5-hydroxy pyridine.

EXAMPLE 12

Preparation of 1-Methyl-2-[[N-phenylaminocarbonyl]hydrazono]methyl pyridinium chloride (3d).

2-[[N-Phenylaminocarbonyl]hydrazono]methyl pyridine. Pyridine-2-Aldehyde (5.7 mL, 0.06 mol) was added to a suspension of sodium acetate (4.92 g, 0.06 mol) and 4-phenyl semicarbazide (9.07 g, 0.06 mol) in 60 mL of water. The resulting mixture was stirred at room temperature for 14 h and the solid in suspension was filtered and washed thoroughly with water. After drying, the semicarbazone was

obtained as a dark beige solid (12.54 g, 87%) and was used in the next step without further purification; m.p. 181°–182° C.

1-Methyl-2-[[N-phenylaminocarbonyl]hydrazono]methyl pyridinium chloride. 2-[[N-Phenylaminocarbonyl]hydrazono]methyl pyridine (4.47 g, 0.02 mol) was divided into two pressure glass tubes and dissolved in acetonitrile (20 mL each). Methyl iodide was then added (2.6 mL each, 0.04 mol), the tubes were tightly closed and heated at 65° C. for 3 days. The reaction flasks were cooled to 10°–15° C. and the solid was filtered. The yellow solid was washed with acetone and ether and dried to yield 6.66 g (92%) of the iodide salt; m.p. 192°–193° C. (dec.). It was dissolved in 400 mL of acetonitrile/water 1:1 and exchanged with freshly made AgCl (from 5.92 g of AgNO₃ and excess conc. HCl, washed well with water). The resulting suspension was stirred at room temperature for 2 h. The silver iodide was filtered off and the filtrate was concentrated to dryness in vacuo to yield a yellow solid that was triturated with acetone and ether. It was filtered and dried to yield 4.86 (96%) of the pure chloride salt as a yellow solid; m.p. 187°–188° C. (dec.). ¹H NMR (d₆-DMSO) δ: 11.87 (s, 1H); 9.45 (s, 1H); 8.98 (d of d, 2H); 8.56 (t, 1H); 8.43 (s, 1H); 8.01 (t, 1H); 7.62 (d, 2H); 7.34 (t, 2H); 7.08 (t, 1H); 4.36 (s, 3H). Anal. Calcd. for C₁₄H₁₅ClN₄O x 0.48 H₂O: C, 56.15; H, 5.37; N, 18.71; Cl, 11.84. Found: C, 56.15; H, 5.38; N, 18.69; Cl, 11.88.

EXAMPLE 13

Preparation of 2-[[Aminocarbonyl]hydrazono]methyl-3-(N,N-dimethylcarbamoyl)hydroxy-1-methyl pyridinium chloride (4a).

2-[[Aminocarbonyl]hydrazono]methyl-3-hydroxy pyridine. Sodium acetate (2.21 g, 16 mmol) was dissolved in 25 mL of water and then semicarbazide hydrochloride was added (1.81 g, 16 mmol) followed by 3-hydroxy-2-pyridine aldehyde (2.0 g, 16 mmol). The resulting mixture was stirred at room temperature for 2 h. The solid in the reaction mixture was then filtered, washed with water and dried to yield 2.71 g (93%) of the semicarbazone. It was used in the next step without further purification; m.p.: 215°–217° C. (dec.).

2-[[Aminocarbonyl]hydrazono]methyl-3-(N,N-dimethylcarbamoyl)hydroxy pyridine. The semicarbazone obtained in the previous step (2.71 g, 15 mmol) was suspended in 20 mL of pyridine and then N,N-dimethylcarbamyl chloride was added (4.2 mL, 45 mmol). The reaction flask was sealed with a septum and the mixture was stirred at room temperature for 20 h. The reaction mixture becomes a clear solution after one hour and the product begins to come out of solution after 2 h. When the reaction is complete, the mixture is cooled to 5°–10° C. in ice bath and filtered. The beige solid that results is washed with acetone/ether 1:1 and dried to yield 3.63 g (? 6%) of the carbamate which is used in the next step without further purification; m.p. 182°–3° C. (dec.).

2-[[Aminocarbonyl]hydrazono]methyl-3-(N,N-dimethylcarbamoyl)hydroxy-1-methyl pyridinium chloride. The carbamate obtained in the previous step (3.63 g, 14 mmol) was dissolved in 50 mL hot (90° C.) DMF. When a clear solution was obtained, the reaction flask was cooled under running water until the inner temperature reached 26° C. Methyl iodide (6.3 mL, 100 mmol) was then added and the reaction mixture was left at 65° C. in a Parr shaker for 3 days. The solvent was removed in vacuo to yield a dark viscous residue which was triturated with acetone. The yellow solid that resulted was washed with acetone/ether

1:1, then with ether and dried to yield 5.15 g (94%) of the methiodide. Freshly made AgCl (from 4.45 g AgNO₃ and excess conc. HCl) was washed well with water and added to a solution of the methiodide (5.15 g, 13 mmol) in 200 mL acetonitrile/water 1:1. The resulting suspension was stirred at room temperature for 1.5 h. The silver iodide was filtered through Celite and the filtrate was concentrated to dryness in vacuo to yield a residue which is triturated with acetone. The resulting solid is filtered and recrystallized from CH₃CN/EtOH and acetone to yield 2.44 g (62%) of the pure chloride salt; m.p. 194°–195° C. (dec.). Anal. Calcd. for C₁₁H₁₆ClN₅O₃ x 0.96 H₂O: C, 41.42; H, 5.66; N, 21.96; Cl, 111.12. Found: C, 41.42; H, 5.72; N, 21.81; Cl, 111.20.

2-[(Aminocarbonyl)hydrazono]methyl-3-(N,N-dimethylcarbamoyl)hydroxy-1,6-dimethyl pyridinium chloride can be made by the same procedure replacing 3-hydroxy-2-pyridine aldehyde in the above example with 3-hydroxy-6-methyl-2-pyridine aldehyde. 2-[(Aminocarbonyl)hydrazono]methyl-5-(N,N-dimethylcarbamoyl)hydroxy-1-methyl pyridinium chloride can be made by the same procedure replacing 3-hydroxy-2-pyridine aldehyde in the above example with 2-formyl-5-hydroxy pyridine.

EXAMPLE 14

Preparation of 3-(N,N-Dimethylcarbamoyl)hydroxy-1-methyl-2-[(N-phenylaminocarbonyl) hydrazonolmethyl] pyridinium chloride (4b).

3-(N,N-Dimethylcarbamoyl)hydroxy-2-[(N-phenylaminocarbonyl)hydrazono]methyl pyridine was prepared by reaction of dimethylcarbamyl chloride (16 mL, 0.18 mol) with 3-hydroxy-2-[(N-phenylaminocarbonyl)hydrazono]methyl pyridine (4.5 g, 17.5 mmol) in 30 mL of pyridine. The resulting mixture was stirred at room temperature for 6 h and poured over 500 mL of crashed ice. The beige precipitate was collected, washed thoroughly with water and dried to yield 5.48 g (95%) of product, m.p. 178°–180° C. The product (5.48 g, 16.7 mmol) was dissolved in acetonitrile/DMF and then methyl iodide (2.78 mL, 44.7 mmol x 3 vessels) was added to the reaction vessel. The resulting mixture was stirred at 70° C. for 3 days and cooled in a water-ice bath for 30 min. The precipitate was collected by filtration, washed with acetone/ether 1:1 and dried to yield 6.42 g (82%) of the methiodide salt. It was dissolved in 500 mL H₂O and 300 mL acetonitrile and then freshly made AgCl (2 eq, washed well with water) was added. The resulting suspension was stirred at room temperature for 2h. It was filtered through Celite and the filtrate was concentrated to dryness in vacuo to a crisp yellow foam that becomes a yellow solid after trituration with acetone. The yellow solid was filtered, washed with ether and dried to yield 4.83g (93%) of pure product as the chloride salt; m.p. 176°–177° C. (dec.). Anal. Calcd. for C₁₇H₂₀ClN₅O₃ x 0.84 H₂O: C, 51.95; H, 5.56; N, 17.82; Cl, 9.02. Found: C, 51.95; H, 5.55; N, 17.80; Cl, 19.11.

5-(N,N-Dimethylcarbamoyl)hydroxy-1-methyl-2-[(N-phenylaminocarbonyl) hydrazono]methyl pyridinium chloride can be made by the same procedure replacing 3-hydroxy-2-pyridine aldehyde with 2-formyl-5-hydroxy pyridine.

EXAMPLE 15

Preparation of 2-(3-Hydroxy-1-methyl-2-pyridinemethylene)-1-[2-(1-pyridinium)acetyl]hydrazine dichloride (5a).

2-(3-Hydroxy-2-pyridinemethylene)-1-[2-(1-pyridinium)acetyl] hydrazine chloride. 1-(Carboxymethyl) pyridinium

chloride hydrazide (2.63 g, 0.014 mol) in 10 mL of methanol was added to a solution of 2-aldehyde-3-hydroxy pyridine (1.72 g, 0.014 mol) in 20 mL of CH₃CN. The resulting dark yellow solution is stirred at room temperature for 2 h and at the end of this period a beige solid filled the reaction mixture. Ethyl ether was added (10 mL) and the reaction mixture was cooled to 5°–10° C. before filtering the beige precipitate. The solid was washed with ethyl ether and dried to yield 3.72 g (91%) of product; m.p. 229°–30° C. (dec).

2-(3-Hydroxy-1-methyl-2-pyridinemethylene)-1-[2-(1-pyridinium)acetyl] hydrazine dichloride. The acyl hydrazine obtained in the previous step (3.5 g, 0.012 mol) was divided into three pressure glass vessels and each portion was dissolved in 20 mL of acetonitrile. Methyl iodide was added to these solutions (1.2 mL each, 0.02 mol) and the resulting mixtures were heated at 70° C. for 3 days. The reaction mixtures were concentrated in vacuo to a dark residue which was triturated with acetone/ethanol 5:1. The resulting yellow solid was washed with acetone/ethanol 5:1 and dried to obtain 4.39 g (84%) of the iodide salt, m.p. 193°–94° C. (dec). Freshly made AgCl (from 3.43 g of AgNO₃ and excess conc. HCl) was washed well with water and added to a solution of the iodide salt (4.39 g, 0.01 mol) in 300 mL acetonitrile/water 1:1. The resulting suspension was stirred at room temperature for 2 h. The silver iodide was filtered through Celite and the filtrate was concentrated to dryness in vacuo to yield a solid that was triturated with acetone. The dark beige solid was filtered, washed with acetone and dried to yield 2.89 g (84%) of pure product as the chloride salt; m.p. 230°–31° C. (dec.). Anal. Calcd. for C₁₄H₁₆Cl₂N₄O₂ x 0.96 H₂O: C, 46.65; H, 5.01; N, 15.55; Cl, 119.67. Found: C, 46.65; H, 4.95; N, 16.33; Cl, 20.66.

2-(3-Hydroxy-1-methyl-2-pyridinemethylene)-1-[2-(trimethylammonium)acetyl]hydrazine dichloride, 1-[2-(4-chloro-1-pyridinium)acetyl]-2-(3-hydroxy-1-methyl-2-pyridinemethylene) hydrazine dichloride, 1-[2-(3-carboxy-1-isoquinolinium)acetyl]-2-(3-hydroxy-1-methyl-2-pyridinemethylene) hydrazine dichloride, 1-[2-(2-carboxy-4-methoxy-1-quinolinium)acetyl]-2-(3-hydroxy-1-methyl-2-pyridine methylene) hydrazine dichloride, 2-(3-hydroxy-1-methyl-2-pyridinemethylene)-1-[2-(3-hydroxy-1-pyridinium)acetyl]hydrazine dichloride, 2-(3-hydroxy-1-methyl-2-pyridinemethylene)-1-[2-(1-methyl-1-piperidinium)acetyl] hydrazine dichloride can be made by the same procedure replacing 1-(carboxymethyl) pyridinium chloride hydrazide respectively with (carboxymethyl) trimethylammonium chloride hydrazide (Girard's reagent "T"), 4-chloro-1-(carboxymethyl)pyridinium chloride hydrazide, 3-carboxy-1-(carboxymethyl)isoquinolinium chloride hydrazide, 2-carboxy-4-methoxy-1-(carboxymethyl)quinolinium chloride hydrazide, 3-hydroxy-1-(carboxymethyl)pyridinium chloride hydrazide, and 1-methyl-1-(carboxymethyl) piperidinium chloride hydrazide. 2-(5-Hydroxy-1-methyl-2-pyridinemethylene)-1-[2-(1-pyridinium)acetyl] hydrazine dichloride, 2-(5-hydroxy-1-methyl-2-pyridinemethylene)-1-[2-(trimethylammonium)acetyl]hydrazine dichloride, 1-[2-(4-chloro-1-pyridinium)acetyl]-2-(5-hydroxy-1-methyl-2-pyridinemethylene) hydrazine dichloride, 1-[2-(3-carboxy-1-isoquinolinium)acetyl]-2-(5-hydroxy-1-methyl-2-pyridinemethylene) hydrazine dichloride, 1-[2-(2-carboxy-4-methoxy-1-quinolinium)acetyl]-2-(5-hydroxy-1-methyl-2-pyridinemethylene) hydrazine dichloride, 2-(5-hydroxy-1-methyl-2-pyridinemethylene)-1-[2-(3-hydroxy-1-pyridinium)acetyl]hydrazine dichloride, and 2-(5-hydroxy-1-methyl-2-pyridinemethylene)-1-[2-(1-piperidinium)acetyl] hydrazine dichloride can be made by the same

procedure replacing 3-hydroxy-2-pyridine aldehyde with 2-formyl-5-hydroxy pyridine.

EXAMPLE 16

Preparation of 2-(1-Methyl-2-pyridinemethylene)-1-[2-(1-pyridinium)acetyl] hydrazine dichloride (5b).

2-(2-pyridinemethylene)-1-[2-(1-pyridinium)acetyl] hydrazine chloride. 1-(Carboxymethyl) pyridinium chloride hydrazide (5.25 g, 28 mmol) and pyridine-2-carbaldehyde (2.7 mL, 28 mmol) were dissolved in 25 mL acetonitrile:methanol (4: 1). The resulting mixture was stirred at room temperature for 2 h, with the product beginning to come out of solution after 20 min of reaction. After 2 h the white solid in the reaction mixture was filtered and washed with acetonitrile and ether. After drying, the product was obtained as a white solid (5.27 g, 68%) and was used in the next step without further purification; m.p. 240°–241° C.(dec.). ¹H NMR (d₆-DMSO) δ: 12.43 (br.s., 1H); 9.07 (d, 2H); 8.70 (t, 1H); 8.66 (d, 1H); 8.24 (d old, 2H); 8.19 (s, 1H); 8.01–7.90 (m, 2H); 7.48–7.44 (m, 1H); 6.07 (s, 2H).

2-(1-Methyl-2-pyridinemethylene)-1-[2-(1-pyridinium)acetyl] hydrazine dichloride. The acyl hydrazone obtained in the previous step (2.88 g, 10 mmol) was divided into two pressure vessels and each portion was dissolved in 25 mL of DMF. Methyl iodide was added to these solutions (1.62 mL each, 25 mmol) and the resulting mixtures were heated at 70° C. for 3 days. The solvent was removed in vacuo to yield a dark residue which was triturated with acetone. The resulting solid was filtered, washed with acetone and ether and dried to yield 4.25 g (quantitative) of the methiodide as a yellow solid. Freshly made AgCl (from 3.77 g AgNO₃ and excess cone. HCl) was washed well with water and added to a solution of the iodide salt (4.18 g, 10 mmol) in 200 mL acetonitrile/water 1:1. The resulting suspension was stirred at room temperature for 1.5 h. The silver iodide was filtered through Celite and the filtrate was concentrated to dryness in vacuo to yield 3.63 g of a yellow solid. Recrystallization from ethanol/acetone affords the pure chloride as a beige solid (3.0 g, 92%); m.p. 209°–210° C.(dec.). ¹H NMR (d₆-DMSO) δ: 9.13 (d, 2H); 9.08 (d, 1H); 8.75–8.59 (m, 4H); 8.26 (t, 2H); 8.10 (m, 1H); 6.19 (br.s., 2H); 4.42 (s, 3H). Anal. Calcd. for C₁₄H₁₆Cl₂N₄O x 0.91 H₂O C, 48.94; H, 5.23; N, 16.31; Cl, 20.64. Found: C, 48.94; H, 5.25; N, 16.31; Cl, 20.43.

EXAMPLE 17

Preparation of O-Diphenylmethyl-N-(3-hydroxy-1-methyl-2-pyridinemethylene) Hydroxylamine Iodide.

O-Diphenylmethyl-N-(3-hydroxy-2-pyridinemethylene) hydroxylamine. O-Diphenyl methyl hydroxylamine hydrochloride (3.35 g, 14.2 mmol) was added to a solution of NaOH (0.57 g, 14.2 mmol) in 10 mL of H₂O. After the addition of 10 mL of EtOH, 3-hydroxy-2-pyridine aldehyde (1.75 g, 14.2 mmol) was added. The mixture was heated on a steam bath for 1 h. While the solution was allowed to cool an additional 30 mL of H₂O was added. The product precipitated out as yellow needles. The mixture was filtered to isolate the pure product (3.8 g, 89%), m.p. 83°–84° C. ¹H NMR (CDCl₃) δ: 9.57 (s, 1H); 8.40 (s, 1H); 7.33 (s, 10H); 7.13 (m, 3H); 6.23 (s, 1H). Anal. Calcd. for C₁₉H₁₆N₂O₃: C, 74.97; H, 5.31; N, 9.21. Found: C, 74.88; H, 5.32; N, 9.17.

O-Diphenylmethyl-N-(3-hydroxy-1-methyl-2-pyridinemethylene) hydroxylamine iodide. A solution of O-diphenylmethyl-N-(3-hydroxy-2-pyridinemethylene) hydroxylamine (1.0 g, 3.29 mmol) in 45 mL of EtOH was placed in a glass pressure tube. Methyl iodide (0.9 mL, 13.8

mmol) was then added, and the tube was sealed tightly. The tube was heated at 69° C. in an oil bath for 20 h. The solvent was removed in vacuo, and the residue was triturated with acetone to give the product as a shiny dark yellow solid (1.4 g, 95%), m.p. 163°–165° C. The chloride salt can be obtained as described above.

EXAMPLE 18

Preparation of O-Benzyl-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) Hydroxylamine Chloride.

3-Hydroxy-6-methyl-2-pyridine carbaldehyde. 3-Hydroxy-6-methyl-2-pyridine methanol (20.0 g, 0.14 mol) and selenium dioxide (8.0 g, 72 mmol) were dissolved in 140 mL of 1,4-dioxane and 280 mL of absolute ethanol. The resulting mixture was heated at 80°–85° C. for 12 h. The selenium precipitate was removed by filtration and the filtrate was concentrated to dryness in vacuo. The dark red residue was sublimed at 90° C. and 14 mmHg for 6 h to obtain 11.0 g (56%) of pure product, m.p. 101°–102° C.; ¹H NMR (CDCl₃) δ: 10.50 (br s, 1H), 10.03 (s, 1H), 7.27 (s, 2H); 2.53 (s, 3H).

O-Benzyl-N-(3-hydroxy-6-methyl-2-pyridinemethylene) hydroxylamine. Sodium hydroxide (3.2 g, 0.08 mol) was dissolved in 80 mL of a 1:1 mixture of ethanol/water and then O-Benzylhydroxylamine hydrochloride (12.77 g, 0.08 mol) was added. The reaction mixture was stirred at room temperature and when a clear solution was obtained 11.0 g (0.08 mol) of 3-hydroxy-6-methyl-2-pyridine carbaldehyde was added and the resulting mixture was stirred at room temperature for 1 h. The reaction mixture was cooled in an ice bath and filtered to yield 18.53 g (96%) of product as a white solid, m.p. 81°–82° C. ¹H NMR (Me₂SO-d₆) δ: 9.85 (br s, 1H), 8.40 (s, 1H), 7.30–7.47 (m, 5H), 7.22 (d, 1H), 7.13 (d, 1H), 5.22 (s, 2H), 2.36 (s, 3H).

O-Benzyl-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydroxylamine iodide. O-Benzyl-N-(3-hydroxy-6-methyl-2-pyridinemethylene) hydroxylamine (10.0 g, 0.04 mol) was dissolved in 75 mL of acetonitrile and 7.7 mL (0.12 mol) of methyl iodide. The reaction mixture was heated in a pressure glass vessel at 90° C. for 3 days. The solvent was removed in vacuo and the residue was triturated with acetone to yield 8.1 g of a yellow solid. Recrystallization from acetone/EtOH/Et₂O yields 5.5 g (35%) of pure product as a pale yellow solid, ¹H NMR (Me₂SO-d₆) δ: 8.55 (s, 1H); 7.92 (d, 1H); 7.87 (d, 1H); 7.30–7.48 (m, 5H); 5.2, 5.33 (s, 2H); 3.93, 4.08 (s, 3H); 2.72 (s, 3H).

O-Benzyl-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydroxylamine chloride. O-Benzyl-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydroxylamine iodide (5.5 g, 14 mmol) were dissolved in 700 mL of water and 4.1 g (28 mmol) of freshly prepared AgCl (from silver nitrate and excess cone. HCl) was then added. The suspension was stirred mechanically for 2.5 h at room temperature and the silver iodide was then removed by filtration. The filtrate was concentrated to dryness in vacuo and the residue was triturated with acetone to yield 3.6 g (86%) of pure product as a light beige solid, m.p. 175°–6° (dec.). ¹H NMR (Me₂SO-d₆) δ: 8.52, 8.06 (s, 1H); 8.10 (d, 1H); 7.85 (t, 1H); 7.30–7.45 (m, 5H); 5.30, 5.20 (s, 2H); 4.1, 3.9 (s, 3H); 2.69 (s, 3H). Anal. Calcd. for C₁₅H₁₇ClN₂P₂ x 0.15 H₂O C, 60.96; H, 5.90; N, 9.48; Cl, 12.00. Found: C, 60.96; H, 5.90; N, 9.51; Cl, 12.02.

O-(1-Naphtylmethyl)-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydroxylamine chloride. O-(2-methyl-1-naphtylmethyl)-N-(1,6-dimethyl-3-hydroxy-2-

pyridinemethylene) hydroxylamine chloride, O-methyl-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydroxylamine chloride, O-(p-chlorobenzyl)-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydroxylamine chloride, O-(p-cyanobenzyl)-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydroxylamine chloride, O-(2-hydroxy-4-nitrobenzyl)-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydroxylamine chloride, O-(p-methoxybenzyl)-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydroxylamine chloride, O-(p-dimethylaminocarbonyloxybenzyl)-N-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene) hydroxylamine chloride, O-(p-propylaminocarbonyl benzyl)-N-(1,6-dimethyl-3-hydroxy-2-pyridine methylene) hydroxylamine chloride, O-benzyl-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1,6-dimethyl-2-pyridinemethylene] hydroxylamine chloride, O-benzyl-N-[3-(N',N'-diethylcarbamoyl)hydroxy-1,6-dimethyl-2-pyridinemethylene] hydroxylamine chloride, O-benzyl-N-[3-(N',N'-difluoroethylcarbamoyl)hydroxy-1,6-dimethyl-2-pyridinemethylene] hydroxylamine chloride, O-(diphenylmethyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1,6-dimethyl-2-pyridinemethylene] hydroxylamine chloride, O-(diphenylmethyl)-N-[1,6-dimethyl-3-(N'-isopropylcarbamoyl)hydroxy-2-pyridinemethylene] hydroxylamine chloride, O-(diphenylmethyl)-N-[3-(N'-2-fluoroethyl)carbamoyl]hydroxy-1,6-dimethyl-2-pyridinemethylene] hydroxylamine chloride, O-(p-methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1,6-dimethyl-2-pyridinemethylene] hydroxylamine chloride, O-(p-methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-ethyl-6-methyl-2-pyridinemethylene] hydroxylamine chloride, O-(p-methoxybenzyl)-N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1-benzyl-6-methyl-2-pyridinemethylene] hydroxylamine bromide, N-[3-(N',N'-dimethylcarbamoyl)hydroxy-1,6-dimethyl-2-pyridinemethylene]hydroxylamine chloride, 1,6-dimethyl-3-hydroxy-2-[(N-phenylaminocarbonyl)hydrazono] methyl] pyridinium chloride, 1,6-dimethyl-3-hydroxy-2-[(N-phenylaminothiocarbonyl)hydrazono] methyl] pyridinium chloride, 1,6-Dimethyl-2-[(N,N-diphenylaminocarbonyl)hydrazono]methyl]-3-hydroxy pyridinium chloride, 2-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene)-1-[2-(1-pyridinium)acetyl] hydrazine dichloride, 2-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene)-1-[2-(trimethylammonium)acetyl] hydrazine dichloride, and 2-(1,6-dimethyl-3-hydroxy-2-pyridinemethylene)-1-[2-(4-chloro-1-pyridinium)acetyl] hydrazine dichloride can be made by the same procedure replacing 3-hydroxy-2-pyridine carbaldehyde with 3-hydroxy-6-methyl-2-pyridine carbaldehyde.

EXAMPLE 19

Preparation of O-Benzyl-N-(1-benzyl-3-hydroxy-2-pyridinemethylene) hydroxylamine bromide.

O-Benzyl-N-(3-hydroxy-2-pyridinemethylene) hydroxylamine. O-Benzyl hydroxylamine (10.4 g, 65 mmol) was added to a solution of NaOH (2.6 g, 65 mmol) in 66 mL of H₂O/EtOH 1:1. 3-Hydroxy-2-pyridine carbaldehyde (8.0 g, 65 mmol) was added to this solution and the resulting mixture was stirred at room temperature 3 h. The white precipitate was then filtered and dried to yield 13.3 g (90%) of pure product, m.p. 64°–65° C. ¹H NMR (CDCl₃) δ: 9.72 (s, 1H); 8.37 (s, 1H); 8.13 (d of d, J=2Hz, J=4 Hz, 1H); 7.33 (m, 7H); 5.17 (s, 2H).

O-Benzyl-4-(1-benzyl-3-hydroxy-2-pyridinemethylene) hydroxylamine bromide. Benzyl bromide (0.78 mL, 6.6 mmol) was added to a solution of O-benzyl-N-(3-hydroxy-

2-pyridine methylene) hydroxylamine (1.0 g, 4.4 mmol) in 4 mL of acetonitrile. The resulting mixture was heated at 65° C. in a pressure bottle for 3 days. The solvent was removed in vacuo and coevaporated with water to leave an oily residue that was dissolved in 100 mL of water and treated with activated charcoal. This mixture was filtered through Celite and the filtrate was concentrated to dryness under high vacuum. Acetone (20 mL) was added to the residue and the product crystallized as light beige needles (1.5 g, 86%), m.p. 145°–146° C. ¹H NMR (CDCl₃) δ: 8.68 (s, 1H); 8.63 (d of d, 1H); 8.52 (t, 1H); 7.69 (d of d, 1H); 7.35 (m, 8H); 7.10 (m, 2H); 6.12 (s, 2H); 5.25 (s, 2H). Anal. Calcd. for C₂₀H₁₉BrN₂O₂: C, 60.16; H, 4.80; N, 7.02; Br, 20.02. Found: C, 59.89; H, 4.86; N, 7.00; Br, 19.91.

O-Benzyl-N-(1-benzyl-5-hydroxy-2-pyridinemethylene) hydroxylamine bromide can be made by the same procedure replacing 3-hydroxy-2-pyridine aldehyde in the above example with 2-formyl-5-hydroxy pyridine.

GENERAL PROCEDURES

2,6-Dichlorophenylsemicarbazide (General Procedure for Phenylsemicarbazide Synthesis). The synthesis of phenylsemicarbazides were carried out according to procedures by Van Gelderen (1933). To a stirring solution of 10 mL methylene chloride and 2 g (I 1 mmol) hydrazine monohydrate at 0° C. was added dropwise a suspension of 5.5 g (110 mmol) of 2,6-dichlorophenyl isocyanate and 10 mL methylene chloride. The semicarbazide was formed instantaneously and collected by suction filtration as a white solid. The product was washed with 100 mL water and 100 mL methylene chloride; yield 600 mg (25%); mp 288°–290° C. ¹H NMR (DMSO-d₆) δ 8.30–8.26 (bs, 1H), 7.55 (s, 1H), 7.50 (dd, 2H), 7.23 (m, 1H), 4.42–4.40 (bs, 2H); FABMS m/e (M+1)⁺ 220.1. Anal. calcd. for C₇H₄N₃OCl₂: C, 38.18; H, 3.20; N, 19.10; Cl, 132.23. Found: C, 38.04; H, 3.09; N, 18.93; Cl, 32.40.

4-Methoxyphenylsemicarbazide. This compound was prepared by the general procedure and collected as a white solid; yield 39%; mp 150°–152° C. ¹H NMR (DMSO-d₆) δ: 8.62 (s, 1H), 7.44 (d, 2H), 7.41 (s, 1H), 6.87 (d, 2H), 4.33 (s, 2H), 3.69 (s, 3H); g FABMS m/e (M+1)⁺ 182.

4-Chlorophenylsemicarbazide. This compound was prepared by the general procedure and collected as a white solid; yield 80%; mp 260°–262° C. ¹H NMR (DMSO-d₆) δ: 8.80 (s, 1H), 7.60 (d, 2H), 7.50 (s, 1H), 7.78 (d, 2H), 4.39–4.30 (bs, 2H); High resolution MS, m/e (M⁺) calcd. for C₇H₆N₃OCl 185.035. Found: 185.035. Found: 185.035.

2,6-Dimethylphenylsemicarbazide. This compound was prepared by the general procedure and collected as a white solid; yield 77%; mp 270°–272° C. ¹H NMR (DMSO-d₆) δ: 7.85 (s, 1H), 7.24 (s, 1H), 7.03 (m, 3H), 4.40–4.33 (bs, 2H), 2.18 (s, 6H); High resolution MS, m/e (M⁺) calcd. for C₉H₁₃N₃O 179.105. Found: 179.106.

2,6-Difluorophenylsemicarbazide. This compound was prepared by the general procedure except that the product was washed with 50 mL H₂O, 50 mL ethyl acetate, and 50 mL ether, and collected as a white solid; yield 73%; mp 156°–158° C. ¹H NMR (DMSO-d₆) δ: 8.14 (s, 1H), 7.55 (s, 1H), 7.28 (m, 1H), 7.11 (dd, 2H), 4.42–4.39 (bs, 2H); FABMS m/e (M+1)⁺ 188.

2-Ethoxyphenylsemicarbazide. This compound was prepared by the general procedure and collected as a white solid; yield 93%; mp 133°–135° C. ¹H NMR (DMSO-d₆) δ: 9.00–8.90 (bs, 1H), 8.18 (m, 1H), 7.63 (s, 1H), 6.97 (m, 1H), 6.87 (m, 2H); 4.47–4.51 (bs, 2H), 4.12 (q, 2H), 1.38 (t, 3H); MS m/e (M⁺) 195.1. Anal. calcd. for C₉H₁₃N₃O₂: C, 55.37; H, 6.71; N, 21.52. Found: C, 55.38; H, 6.74; N, 21.47.

3-Nitrophenylsemicarbazide. This compound was prepared by the general procedure and collected as a yellow solid; yield 59%; mp 136°–138° C. ¹H NMR (DMSO-*d*₆) δ: 9.21 (s, 1H), 8.68 (s, 1H), 7.89 (d, 1H), 7.76 (m, 2H), 7.5 (m, 1H), 4.44–4.38 (bs, 2H); High resolution, MS *m/e* (*M*⁺) calcd. for C₇H₆O₃N₄ 196.059. Found: 196.058.

4-Bromophenylsemicarbazide. This compound was prepared by the general procedure and collected as a white solid; yield 85%; mp 256°–259° C. ¹H NMR (DMSO-*d*₆) δ: 8.78 (s, 1H), 7.48–7.55 (d and bs, 3H), 7.40 (d, 2H), 4.40–4.37 (bs, 2H); High resolution, MS *m/e* (*M*⁺) calcd. for C₇H₆N₃OBr 228.985. Found: 228.984.

1-(3-Hydroxy-2-pyridylmethylene)-4-(2,6-dichlorophenyl) diazanecarboxamide (General Procedure for the Condensation of Phenylsemicarbazides or Phenylhydrazides with Pyridine Aldehydes). A solution of 500 mg (4 mmol) of 3-hydroxy-2-pyridinecarboxaldehyde and 876 mg (4 mmol) of 2,6-dichlorophenylsemicarbazide in 20 mL of ethanol was refluxed for 1 h. The mixture was cooled, filtered by gravity, washed several times with 25 mL portions of ethanol, and air dried. The product was obtained as a brown solid; yield 1.20 g (95%); mp 262°–265° C. ¹H NMR (DMSO-*d*₆) δ: 11.05 (s, 1H), 10.28–10.10 (bs, 1H), 9.05 (s, 1H), 8.30 (s, 1H), 8.18 (d, 1H), 7.58 (dd, 2H), 7.53 (m, 3H); MS *m/e* (*M*⁺) 324.1.

The products of these reactions can be made in purer forms by the addition of 1 drop of H₂SO₄ to the reaction mixture before refluxing as in the case of 1-(3-hydroxy-2-pyridylmethylene)-4-(2,6-dimethylphenyl) diazanecarboxamide given below.

1-(3-Hydroxy-2-pyridylmethylene)-4-(4-methoxyphenyl) diazanecarboxamide. This compound was prepared by the general procedure except that the crude product was dissolved in hot methanol, concentrated in vacuo, and collected as a brown solid; yield 47%; mp 183°–185° C. ¹H NMR (DMSO-*d*₆+D₂O) δ: 9.05 (s, 1H), 8.44 (s, 1H), 8.20 (s, 1H), 7.5 (d, 2H), 7.4 (s, 1H), 7.3 (d, 2H), 6.8 (d, 2H), 3.7 (s, 3H); FABMS *m/e* (*M*+1)⁺ 287.

1-(3-Hydroxy-2-pyridylmethylene)-2-(2,4,6-trichlorophenyl) diazane. This compound was prepared by the general procedure. After cooling the product was obtained as crispy white needles; yield 84%; mp 185°–187° C. ¹H NMR (DMSO-*d*₆) δ: 10.59 (s, 1H), 10.20 (s, 1H), 8.36 (s, 1H), 8.14 (d, 1H), 7.7 (s, 1H), 7.24 (m, 2H); MS *m/e* (*M*⁺) 315.1.

1-(3-Hydroxy-2-pyridylmethylene)-4-(4-chlorophenyl) diazanecarboxamide. This compound was prepared by the general procedure. The product was a brown solid; yield 33%; mp 216°–218° C. ¹H NMR (DMSO-*d*₆) δ: 10.96 (s, 1H), 10.60–10.50 (bs, 1H), 9.28 (s, 1H), 8.29 (s, 1H), 8.17 (d, 1H), 7.62 (dd, 2H), 7.2 (m, 4H); MS *m/e* (*M*⁺) 290.1.

1-(3-Hydroxy-2-pyridylmethylene)-4-(2,6-dimethylphenyl) diazanecarboxamide. This compound was prepared by the general procedure. The product was a brown solid; yield 87%; mp 243°–248° C. ¹H NMR (DMSO-*d*₆) δ: 10.81 (s, 1H), 10.40–10.30 (bs, 1H), 8.53 (s, 1H), 8.27 (s, 1H), 8.16 (d, 1H), 7.33 (m, 2H), 7.10 (s, 3H), 2.20 (s, 6H); FABMS *m/e* (*M*+1)⁺ 285.

1-(3-Hydroxy-2-pyridylmethylene)-4-(2,6-difluorophenyl) diazanecarboxamide. This compound was prepared by the general procedure. The product was a brown solid; yield 56%; mp 238°–240° C. ¹H NMR (DMSO-*d*₆) δ: 11.06 (s, 1H), 10.05–10.15 (bs, 1H), 8.88 (s, 1H), 8.31 (s, 1H), 8.18 (s, 1H), 7.35 (m, 3H), 7.18 (m, 2H); MS *m/e* (*M*⁺) 292.

1-(3-Hydroxy-2-pyridylmethylene)-4-(2-ethoxyphenyl) diazanecarboxamide. This compound was prepared by the

general procedure. The product was a brown solid; yield 58%; mp 197°–198° C. ¹H NMR (DMSO-*d*₆) δ: 11.14 (s, 1H), 10.70–10.90 (bs, 1H), 8.73 (s, 1H), 8.27 (s, 1H), 8.15 (m, 2H), 7.32 (m, 2H), 7.05 (m, 3H), 4.13 (q, 2H), 1.45 (t, 3H); MS *m/e* (*M*⁺) 300.2.

1-(3-Hydroxy-2-pyridylmethylene)-2-(4-fluorophenyl) diazane. This compound was prepared by the general procedure. The product was a red solid; yield 76%; mp 260°–264° C. (dec). ¹H NMR (DMSO-*d*₆) δ: 12.15–12.23 (bs, 1H), 12.07 (s, 1H), 8.24 (d, 1H), 8.12 (m, 2H), 7.66 (m, 1H), 7.62 (m, 2H), 7.20 (m, 2H); MS *m/e* (*M*⁺) 231.1.

1-(3-Hydroxy-2-pyridylmethylene)-4-(3-nitrophenyl) diazanecarboxamide. This compound was prepared by the general procedure. The product was a brown solid; yield 73%; mp 223°–225° C. ¹H NMR (DMSO-*d*₆) δ: 11.11 (s, 1H), 10.50–10.30 (bs, 1H), 9.68 (s, 1H), 8.62 (s, 1H), 8.33 (s, 1H), 8.20 (s, 1H), 8.19 (d, 1H), 7.97 (d, 1H), 7.88 (m, 1H), 7.61 (m, 2H); MS *m/e* (*M*⁺) 300.1.

1-(3-Hydroxy-2-pyridylmethylene)-4-(4-bromophenyl) diazanecarboxamide. This compound was prepared by the general procedure. The product was a brown solid; yield 91%; mp 274° C. (dec). ¹H NMR (DMSO-*d*₆) δ: 10.95 (s, 1H), 10.45–10.65 (bs, 1H), 9.29 (s, 1H), 8.30 (s, 1H), 8.19 (d, 1H), 7.50 (dd, 4H), 7.33 (m, 2H); MS *m/e* (*M*+1)⁺ 335.

1-(3-(*N,N*-Dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(2,6-dichlorophenyl) diazanecarboxamide (General Procedure for the Synthesis of Pyridine Carbamates). The synthesis of pyridine carbamates was carried out according to procedures by Powers et al. (1993). A solution of 500 mg (1.5 mmol) of 1-(3-hydroxy-2-pyridylmethylene)-4-(2,6-dichlorophenyl) diazanecarboxamide and 1.65 g (15 mmol) of dimethylcarbonyl chloride in 15 mL of pyridine was allowed to stir for 14 h at room temperature. The reaction mixture was poured over crashed ice and the white solid which formed was washed thoroughly with water to remove the excess pyridine; yield 280 mg (47%); mp 225°–229° C. ¹H NMR (DMSO-*d*₆) δ: 11.17 (s, 1H), 8.53 (d, 1H), 8.16 (s, 1H), 8.12 (s, 1H), 7.7 (d, 1H), 7.5 (d, 2H), 7.4 (dd, 1H), 7.3 (t, 1H), 3.0 (s, 3H), 2.7 (s, 3H); MS *m/e* (*M*⁺) 395.2.

1-(3-(*N,N*-Dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(4-methoxyphenyl) diazinecarboxamide. This compound was prepared by the general procedure and collected as a brown solid; yield 57%; mp 146°–152° C. ¹H NMR (DMSO-*d*₆) δ: 10.91 (s, 1H), 8.52 (d, 1H), 8.34 (s, 1H), 8.09 (s, 1H), 7.67 (d, 1H), 7.46 (m, 3H), 3.73 (s, 3H), 3.08 (s, 3H), 2.85 (s, 3H); MS *m/e* (*M*+1)⁺ 358.

1-(3-(*N,N*-Dimethylcarbamoyloxy)-2-pyridylmethylene)-2-(2,4,6-trichlorophenyl) diazine. This compound was prepared by the general procedure. The crude product recrystallizes from ethanol or methanol into a fluffy yellow solid; yield 58%; mp 145°–147° C. ¹H NMR (DMSO-*d*₆) δ: 8.62 (d, 1H), 7.84 (d, 1H), 7.68 (s, 2H), 7.67 (dd, 1H), 7.47 (s, 1H), 3.14 (s, 3H), 2.95 (s, 3H); MS *m/e* (*M*+1)⁺ 387.

1-(3-(*N,N*-Dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(4-chlorophenyl) diazanecarboxamide. This compound was prepared by the general procedure except that the crude product was dissolved in hot ethyl acetate, concentrated in vacuo and collected as a brown solid; yield 36%; mp 170°–175° C. ¹H NMR (DMSO-*d*₆) δ: 11.02 (s, 1H), 8.82 (s, 1H), 8.52 (d, 1H), 8.12 (s, 1H), 7.68 (m, 3H), 7.47 (dd, 1H), 7.37 (dd, 2H), 3.14 (s, 3H), 2.96 (s, 3H); MS *m/e* (*M*+1)⁺ 362.

1-(3-(*N,N*-Dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(2,6-dimethylphenyl) diazanecarboxamide. This compound was prepared by the general procedure. The product was a brown solid; yield 78%; mp 215°–217° C. ¹H NMR

(DMSO- d_6) δ : 10.97 (s, 1H), 8.52 (d, 1H), 8.10 (s, 1H), 7.73 (s, 1H), 7.66 (d, 1H), 7.46 (m, 1H), 7.11 (s, 3H), 3.00 (s, 3H), 2.71 (s, 3H); MS m/e (M^+) 355.2.

1-(3-(N,N-Dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(2,6-difluorophenyl) diazanecarboxamide. This compound was prepared by the general procedure. The product is a white solid; yield 70%; mp 166°–169° C. ^1H NMR (DMSO- d_6) δ : 11.17 (s, 1H), 8.53 (d, 1H), 8.13 (d, 2H), 7.70 (d, 1H), 7.49 (m, 1H), 7.40 (m, 1H), 7.21 (m, 2H), 3.07 (s, 3H), 2.84 (s, 3H); MS m/e (M^+) 363.1.

1-(3-(N,N-Dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(2-ethoxyphenyl) diazanecarboxamide. This compound was prepared by the general procedure. The product is a white solid; yield 70%; mp 166°–169° C. ^1H NMR (DMSO- d_6) δ : 11.15 (s, 1H), 8.89 (s, 1H), 8.50 (d, 1H), 8.13 (m, 2H), 7.70 (m, 1H), 7.50 (m, 1H), 7.00 (m, 3H), 4.17 (m, 2H), 3.14 (s, 3H), 2.96 (s, 3H), 1.43 (m, 3H); MS m/e ($M+1$)⁺ 372.

1-(3-(N,N-Dimethylcarbamoyloxy)-2-pyridylmethylene)-2-(4-fluorophenyl) diazane. This compound was prepared by the general procedure except that the crude material was washed three times with 50 mL of water and 50 mL of ether, and then dissolved in acetone. The acetone was concentrated in vacuo until the product crystallized in the form of brown prisms; yield 24%; mp 165°–167° C. ^1H NMR (DMSO- d_6) δ : 10.63 (s, 1H), 8.45 (d, 1H), 7.91 (s, 1H), 7.59 (d, 1H), 7.33 (m, 1H), 7.13 (m, 2H), 7.01 (m, 2H), 3.10 (s, 3H), 2.94 (s, 3H); MS m/e ($M+1$)⁺ 303.

1-(3-(N,N-Dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(3-nitrophenyl) diazanecarboxamide. This compound was prepared by the general procedure. The product was washed thoroughly with 100 mL of ether and 100 mL of water and collected as a white solid; yield 90%; mp 195° C. ^1H NMR (DMSO- d_6) δ : 11.21 (s, 1H), 9.47 (s, 1H), 8.68 (s, 1H), 8.52 (d, 1H), 8.17 (s, 1H), 8.03 (d, 1H), 7.89 (d, 1H), 7.59 (m, 1H), 7.50 (m, 1H), 3.11 (s, 3H), 2.90 (s, 3H); FABMS m/e ($M+1$)⁺ 373.

1-(3-(N,N-Dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(4-bromophenyl) diazanecarboxamide. This compound was prepared by the general procedure and collected as a white solid; yield 89%; mp 176°–179° C. ^1H NMR (DMSO- d_6) δ : 11.03 (s, 1H), 8.88 (s, 1H), 8.52 (d, 1H), 8.13 (s, 1H), 7.49 (m, 2H), 7.46 (m, 4H), 3.14 (s, 3H), 2.88 (s, 3H); MS m/e (M^+) 405.

EXAMPLE 20

1-(1-Methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(2,6-dichlorophenyl) diazanecarboxamide Iodide (General Procedure for the Synthesis of Pyridine Methiodides). The synthesis of pyridine methiodides was carried according to the following procedure. A solution of 3 g (9 mmol) of 1-(3-(N,N-Dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(2,6-dichlorophenyl) diazanecarboxamide, 6.39 g (45 mmol) of iodomethane, and 25 mL of DMF were sealed in a glass pressure tube and heated for 36 h at 65° C. The crude methiodide was isolated by evaporation of the DMF under reduced pressure and trituration of the residue with a 2:1 mixture of acetone and ether. The crude product was recrystallized from ethanol and collected as orange prisms; yield 0.69 g (19%); mp 132°–140° C. ^1H NMR (DMSO- d_6) δ : 11.78 (s, 1H), 9.08 (d, 1H), 8.86 (s, 1H), 8.58 (d, 1H), 8.24 (s, 1H), 8.15 (dd, 1H), 7.60 (d, 2H), 7.50 (dd, 1H), 4.54 (s, 3H), 3.13 (s, 3H), 2.95 (s, 3H); FABMS m/e ($M+$) 410. Anal. calcd. for $\text{C}_{17}\text{H}_{18}\text{N}_5\text{O}_3\text{Cl}_2\text{I}\cdot\text{H}_2\text{O}$: C, 36.71; H, 3.62; N, 12.59. Found: C, 36.67; H, 3.62; N, 12.51.

EXAMPLE 21

1-(1-Methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(4-methoxyphenyl) diazanecarboxam-

ide Iodide. This compound was prepared by the general procedure. The crude methiodide was precipitated by the addition of acetone, recrystallized from acetonitrile, and collected as orange prisms; yield 56%; mp 192°–194° C. ^1H NMR (DMSO- d_6) δ : 11.58 (s, 1H), 9.1 (d, 1H), 8.79 (s, 1H), 8.55 (d, 1H), 8.18 (m, 2H), 7.46 (d, 2H), 6.89 (d, 2H), 4.51 (s, 3H), 3.73 (s, 3H), 3.09 (s, 3H), 2.92 (s, 3H); FABMS m/e (M^+) 372.2. Anal. calcd. for $\text{C}_{18}\text{H}_{22}\text{N}_5\text{O}_4\text{I}$: C, 43.29; H, 4.44; N, 14.02. Found: C, 43.16; H, 4.47; N, 14.03.

EXAMPLE 22

1-(1-Methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-2-(2,4,6-trichlorophenyl) diazane Iodide. This compound was prepared by the general procedure. The crude methiodide was precipitated by the addition of acetone, recrystallized from ethanol, and collected as yellow needles; yield 90%; mp 198°–200° C. ^1H NMR (DMSO- d_6) δ : 11.22 (s, 1H), 8.87 (d, 1H), 8.41 (d, 1H), 8.39 (s, 1H), 7.94 (dd, 1H), 7.85 (s, 2H), 4.3 (s, 3H), 2.89 (s, 3H), 2.83 (s, 3H); FABMS m/e (M^+) 401. Anal. calcd. for $\text{C}_{16}\text{H}_{16}\text{N}_4\text{O}_2\text{Cl}_3\text{I}$: C, 36.28; H, 3.04; N, 10.57; Cl, 20.08; I, 23.96. Found: C, 36.35; H, 3.07; N, 10.53; Cl, 20.04; I, 23.91.

EXAMPLE 23

1-(1-Methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(4-chlorophenyl) diazanecarboxamide Iodide. This compound was prepared by the general procedure except that the crude methiodide was recrystallized from boiling methanol and collected as brown needles; yield 75%; mp 181°–183° C. ^1H NMR (DMSO- d_6) δ : 10.63 (s, 1H), 8.04 (s, 1H), 7.98 (d, 1H), 7.55 (d, 1H), 7.11 (m, 2H), 6.57 (d, 2H), 6.34 (d, 2H), 2.02 (s, 3H), 1.87 (s, 3H), 1.45 (s, 3H); FABMS m/e (M^+) 376. Anal. calcd. for $\text{C}_{17}\text{H}_{16}\text{N}_5\text{O}_3\text{ClI}$: C, 40.53; H, 3.80; N, 13.90; Cl, 17.04; I, 25.20. Found: C, 40.64; H, 3.82; N, 13.91; Cl, 17.01; I, 25.09.

EXAMPLE 24

1-(1-Methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(2,6-dimethylphenyl) diazanecarboxamide Iodide. This compound was prepared by the general procedure except that the crude methiodide was precipitated by the addition of 20 mL of ethyl acetate and 100 mL of water to the crude residue. The product was recrystallized from methanol and collected as orange prisms; yield 23%; mp 214°–217° C. ^1H NMR (DMSO- d_6) δ : 11.71 (s, 1H), 8.99 (d, 1H), 8.58 (d, 1H), 8.40 (s, 1H), 8.31 (s, 1H), 8.23 (m, 1H), 7.12 (s, 3H), 4.54 (s, 3H), 3.12 (s, 3H), 2.93 (s, 3H), 2.19 (s, 6H); FABMS m/e (M^+) 370. Anal. calcd. for $\text{C}_{19}\text{H}_{24}\text{N}_5\text{O}_3\text{I}\cdot\text{H}_2\text{O}$: C, 30.36; H, 3.22; N, 9.32. Found: C, 30.68; H, 3.15; N, 9.36.

EXAMPLE 25

1-(1-Methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(2,6-difluorophenyl) diazanecarboxamide Iodide. This compound was prepared by the general procedure except that ice was added to the residue to precipitate the crude methiodide. The product was recrystallized from ethanol and collected as a red solid; yield 21%; mp 115°–118° C. ^1H NMR (DMSO- d_6) δ : 11.79 (s, 1H), 9.01 (d, 1H), 8.68 (s, 1H), 8.60 (d, 1H), 8.34 (s, 1H), 8.22 (m, 1H), 7.50 (m, 1H), 7.22 (m, 2H), 4.5 (s, 3H), 3.12 (s, 3H), 2.95 (s, 3H); FABMS m/e (M^+) 378. Anal. calcd. for $\text{C}_{17}\text{H}_{18}\text{N}_5\text{O}_3\text{IF}_2\cdot\text{HI}$: C, 32.24; H, 3.02; N, 11.06. Found: C, 32.07; H, 2.88; N, 10.83.

EXAMPLE 26

1-(1-Methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(2-ethoxyphenyl) diazanecarboxamide Iodide. This compound was prepared by the general procedure except that the crude methiodide was precipitated by the addition of ice to the residue. The product was then recrystallized from methanol and collected as yellow prisms; yield 91%; mp 194°–196° C. ¹H NMR (DMSO-d₆) δ: 11.86 (s, 1H), 9.03 (d, 1H), 8.61 (d, 1H), 8.49 (s, 1H), 8.23 (s, 1H), 8.13 (m, 2H), 7.05 (m, 2H), 6.98 (m, 1H), 4.52 (s, 3H), 4.13 (q, 2H), 3.07 (s, 3H), 2.92 (s, 3H), 1.35 (t, 3H); FABMS m/e (M⁺) 386. Anal. calcd. for C₁₉H₂₄N₅O₄I: C, 42.46; H, 4.50; N, 13.03. Found: C, 42.64; H, 4.59; N, 13.03.

EXAMPLE 27

1-(1-Methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-2-(4-fluorophenyl)diazane Iodide. This compound was prepared by the general procedure except that the crude methiodide was precipitated by ice. The product was then recrystallized from ethanol and collected as red prisms; yield, 94%; mp 265°–267° C. ¹H NMR (DMSO-d₆) δ: 11.87 (s, 1H), 8.92 (d, 1H), 8.40 (d, 1H), 8.04 (s, 1H), 7.95 (dd, 1H), 7.21 (m, 4H), 4.42 (s, 3H), 3.06 (s, 3H), 2.98 (s, 3H); FABMS m/e (M⁺) 317. Anal. calcd. for C₁₆H₁₈O₂N₄FL0.5H₂O: C, 42.39; H, 4.22; N, 12.36. Found: C, 42.25; H, 4.22; N, 12.32.

EXAMPLE 28

1-(1-Methyl-B-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(3-nitrophenyl) diazanecarboxamide Iodide. This compound was prepared by the general procedure except that ice was used to precipitate the crude methiodide. The crude product was then dissolved in hot acetone, filtered and refrigerated. The analytically pure product was collected as a yellow solid; yield 25%; mp 200°–202° C. ¹H NMR (DMSO-d₆) δ: 11.84 (s, 1H), 9.52 (s, 1H), 9.07 (d, 1H), 8.64 (d and s, 2H), 8.22 (m, 2H), 8.05 (d, 1H), 7.94 (d, 1H), 7.63 (dd 1H), 4.54 (s, 3H), 3.09 (s, 3H), 2.94 (s, 3H); FABMS m/e 387. Anal. calcd for C₁₇H₁₉O₅N₄I: C, 39.70; H, 3.72; N, 16.34. Found: C, 39.92; H, 3.80; N, 16.13.

EXAMPLE 29

1-(1-Methyl-3-(N,N-dimethylcarbamoyloxy)-2-pyridylmethylene)-4-(4-bromophenyl) diazanecarboxamide Iodide. This compound was prepared by the general procedure except that ice was added to the residue to precipitate the crude methiodide. The crude product was recrystallized from hot methanol and collected red prisms; yield 86%; mp 202° C. ¹H NMR (DMSO-d₆) δ: 11.70 (s, 1H), 9.05 (s, 1H), 9.03 (d, 1H), 8.62 (d, 1H), 8.15 (m, 2H), 7.58 (m, 4H), 4.52 (s, 3H), 3.16 (s, 3H), 2.93 (s, 3H); FABMS m/e (M⁺) 420. Anal. calcd for C₁₇H₁₉N₅O₃BrI: C, 37.24; H, 3.49; N, 12.77. Found: C, 37.42; H, 3.59; N, 12.60.

TABLE I

Kinetic Constants for the Reversible Inhibition of electric Eel and Human Erythrocyte Acetylcholinesterase.

compd	K _i (eel) μM	K _i (human) μM	IC ₅₀ (eel) μM	IC ₅₀ (human) μM
1a	40	100	100	175
1b	500	>600		

TABLE I-continued

Kinetic Constants for the Reversible Inhibition of electric Eel and Human Erythrocyte Acetylcholinesterase.

compd	K _i (eel) μM	K _i (human) μM	IC ₅₀ (eel) μM	IC ₅₀ (human) μM
1c			NT ^a	NT ^a
1d			400	>500
1e			400	>500
1f			NT ^a	NT ^a
3a			325	300
3b	30, 340 ^b			240
4b	85	100		
5a	10	20		
5b	c	c	40	40

^aNT, no inhibition at concentrations up to 1 mM.

^btwo distinct K_i's with eel AChE, multiple binding with both enzymes.

^cMultiple binding observed, only IC₅₀ values determined.

TABLE II

Kinetic Constants for the Irreversible Inhibition of Electric Eel and Human Erythrocyte Acetylcholinesterase.

compd	K _{obs} /[I](eel) (M ⁻¹ s ⁻¹)	K _{obs} /[I] (human) (M ⁻¹ s ⁻¹)	K _i (eel) (μM)
2a	1700	68000	NT ^a
2b	870	1100	NT ^a
2c	5000	>110000	NT ^a
4a	25	2200	100
Pyr ^b	580 ^c	13000	NT ^a
Phy ^d	14000 ^e	19000	NT ^a

^aNT, not tested (inhibition half-life is too short to measure accurately);

^bPyridostigmine;

^cK_{obs}/[I] = 843 M⁻¹s⁻¹ (Forsberg, A. and Pua, G., Eur. J. Biochem. 1984, 140, 153.);

^dPhysostigmine;

^ek_{obs}/[I] = 18500 M⁻¹s⁻¹ (Forsberg, A. and Pua, G., Eur. J. Biochem. 1984, 140, 153.).

TABLE III

In Vivo Evaluation of Pyridinium Derivatives as Intramuscular Pretreatment Agents Against GD in Mice.

compd	LD ₅₀ (mmol/kg)	time ^a (min)	dose ^b (mmol/kg)	% survival
1a	180	15/60	3.0–4.5	10–20
1b	>880	15/60	22	0/10
1d	>320	15/60	9.6	10/20
1e	>760	15/60	320/81	40/10
1f	>610	15/60	310/77	30/20
1g	>514	15/60	16/260	10/20
2a	0.5	15/60	0.009–0.14	80/90
2b	22	15	0.30	90
			1.4	60
			5.4	30
		60	0.30–5.4	60–70
2c	84	15/60	0.005–0.11	50–70
2d	600	15/60	9.0	90/90
			37	90/70
			150	50/0
3b	>40	15/60	1.3/5.2	0/20
3c	>560	15/60	0.017–0.28	40–70/30–40
3d	>430	15/60	0.013–0.215	20–40/10–30
4a	>1240	15/60	0.2	50/90
			0.6	80/100
			2.3	80/80
4b	42	15	0.65	90
			2.7	90
			11	40
4b		60	0.65–11	70–80

TABLE III-continued

In Vivo Evaluation of Pyridinium Derivatives as Intramuscular Pretreatment Agents Against GD in Mice.				
compd	LD ₅₀ (mmol/kg)	time ^a (min)	dose ^b (mmol/kg)	% survival
5a	>670	15/60	18-290	70-100/20-70
5b	380	15/60	0.6	40/20
			24	100/30
			96	100/100

^aCompounds were tested at two different time intervals to allow for differences in bioavailability;

^bThree different doses were tested, only one dose is recorded for inactive compounds.

TABLE IV

In Vivo Evaluation of Pyridinium Derivatives as Oral Pretreatment Agents Against GD in Mice.

compd	LD ₅₀ (mmol/kg)	time ^a (min)	dose ^b (mmol/kg)	% survival
1b	3600	30/120	60	30/70
1e	>3200	30/120	200	0/50
2a	220	30/120	3.4	90/80
2b	490	30/120	120	90/100
2c	140	30/120	2.2	100/100
4b	670	30/120	10	70/100
5a	2900	30/120	730	40/50

^aCompounds were tested at two different time intervals to allow for differences in bioavailability;

^bThree different doses were tested, only one dose is recorded for inactive compounds.

TABLE V

In Vivo Evaluation of Pyridinium Derivatives as Reactivators Against GD in Mice.

compd	LD ₅₀ (mmol/kg)	dose ^a (mmol/kg)	% survival
1a	180	2.8	20
1b	>880	5.6	20
1d	>320	40	20
1e	>760	20	0
1f	>610	12	0
1g	>514	15	0
2a	0.5	0.009-0.14	30-50
2b	22	0.33	20
		1.4	70
		5.4	60
2c	84	1.3	20
3a	>1100	27 ^b	50
		110 ^b	90
		430 ^b	90
		6.8	20
		54	0
		430	70
3b	>40	1.3	10
3c	>560	0.28	10
3d	>430	0.215	20
4a	9.6	0.2-2.3	0-50
4b	42	0.65-11	30-50
5a	>670	18-290	70-90
5b	380	6	20
		24	40
		96	90
2-PAMCl	853	53	0
HI-6	4500	280-1125	90-100

^aThree different doses were tested, only one dose is recorded for inactive compounds;

^badjunct efficacy test, see text for details.

TABLE VI

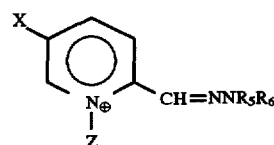
In Vivo Evaluation of Pyridinium Derivatives as Reactivators Against GA in Mice.

compd	LD ₅₀ (mmol/kg)	dose (mmol/kg)	% survival ^a
1c	>1970	30	0
1f	2100	33	0
1g	>510	16	0
2a	0.5	0.009	30
2c	84	0.005	20
4a	9.6	0.60	20
4b	670	2.70	30
5a	>670	18	10
		73	80
		290	90
5b	380	6	10
		24	0
		96	80

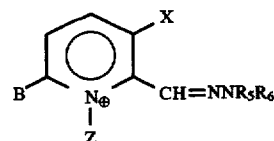
^aThree different doses were tested, only one dose is recorded for inactive compounds.

What is claimed is:

1. A compound of the group consisting of the formulas:



and



and any counterion to make pharmaceutically acceptable salts, wherein

R₅ is selected from the group consisting of pentafluorophenyl, phenyl monosubstituted with K, phenyl disubstituted with K, phenyl trisubstituted with K, and naphthyl.

wherein R₆ is selected from the group consisting of H, CH₁₋₆ alkyl, phenyl, pentafluorophenyl, phenyl mono, di, or trisubstituted with K, CH₁₋₆ alkyl with an attached phenyl group, and C₁₋₆ alkyl with an attached phenyl group mono, di, or trisubstituted with K.

wherein K is selected from the group consisting of halogen, COOH, OH, CN, NO₂, NH₂, C₁₋₆ alkyl, C₁₋₆ fluorinated alkyl, C₁₋₆ alkoxy, C₁₋₆ alkyl-O-CO-, C₁₋₆ alkyl-O-CO-NH-, C₁₋₆ alkyl-NH-CO-NH-, C₁₋₆ alkyl-NH-CO-O-, C₁₋₆ alkyl-NH-CO-, and (C₁₋₆ alkyl)₂N-CO-

wherein X is selected from the group consisting of OH, C₁₋₆ alkyl-NH-CO-O-, (C₁₋₆ alkyl)₂N-CO-O-, C₁₋₆ fluoroalkyl-NH-CO-O-, and (C₁₋₆ fluoroalkyl)₂NH-CO-O-,

wherein B is selected from the group consisting of H, and C₁₋₆ alkyl.

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